# A SIMULATION MODEL OF THE POPULATION DYNAMICS OF THE BLACK SALT MARSH MOSQUITO (*AEDES TAENIORHYNCHUS*) IN A FLORIDA MANGROVE FOREST

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
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Abstract of Dissertation Presented to the Graduate School of the University of Florida in the Partial Fulfillment of the Requirements for the degree of Doctor of Philosophy

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A computer model was developed to simulate the population dynamics of Aedes taeniorhynchus in a mangrove swamp in Collier County, Florida. The model was constructed from data obtained from the literature, from personal communication with authorites and from experiments. A majority of the research involved the egg stage for which the least amount of data was available.

Egg distributions were used to develop a sampling program to collect data for model verification. Highest egg densities occurred in red mangrove leaf litter in a band just above the water level. The eggs were distributed contagiously and fit the negative binomial distribution. Sampling precision was maximized by systematic sampling within an optimal allocation scheme. Sodium hypochlorite facilitated separating and counting eggs in soil and assaying animal feces for egg fragments.

Eggs flushed or hatched when exposed to rain-generated surface runoff. Eggs on compacted soil had significantly higher flushing and hatching rates than did eggs on red mangrove peat. Egg adherence is poor in moist peat and progressively stronger on compacted soil and dry mangrove peat.

Methods were developed to estimate egg mortality in compacted, detrital and flooded soils. Mosquito egg mortality ranged from 10 to 50%/week with highest rates in the summer. Laboratory studies indentified several arthropods that ate mosquito eggs. Submerged eggs developed normally and had mortality rates of ca. 20% per week.

The mosquito simulation model (TAENISIM), written in QUICKBASIC®, realistically simulates Ae. taeniorhynchus population dynamics. Sensitivity analysis indicated that the model is most sensitive to oviposition frequency and stage-specific mortality, especially the adult stage.

Water table wells and staff gauges (which provided a cheaper, more accurate record of water levels) gathered data used to construct a spreadsheet (LOTUS 123®) model to predict mangrove basin water levels used in TAENISIM. TAENISIM, verified using egg and larval population estimates, generally provided accurate predictions of the relative size of egg and larval populations and of the occurrence of hatching, but required the input of migrant adult females to avoid population extinction.

# CHAPTER 1
# INTRODUCTION

Aedes taeniorhynchus (Wiedemann), the black salt marsh mosquito, is the major pest mosquito of coastal Florida. In Collier County (coastal southwest Florida), several million dollars are spent annually to control this mosquito. Three characteristics contribute to the major pest status of this mosquito. First, tremendous populations are common; large broods numbering in the millions of individuals are often produced (Haeger 1960). Second, the mosquito is a fierce biter of man, feeding day and night (Provost 1951). Third, the mosquito often migrates several miles from the larval habitat (Provost 1952), occasionally exposing urban areas to severe mosquito outbreaks (Harden and Chubb 1960). Fortunately, high populations of Ae. taeniorhynchus are generally limited to areas within four miles of the coast (Provost 1951).

Aedes taeniorhynchus is a classic r-strategist species (MacArthur and Wilson 1967). Its high fecundity, rapid development and migratory behavior produce volatile population dynamics (Nayar 1985, Provost 1952). The generalized life cycle of the black salt marsh mosquito, (adapted from Nayar 1985), using summer values, is as follows. Adult females mate within two days of emergence then disperse to

obtain a vertebrate blood meal. Blood allows the mosquito to maximize egg production (clutch size ca. 100), although south Florida Ae. taeniorhynchus often produce an initial clutch (ca. 25 eggs) autogenously (i.e., without a blood meal (O'Meara and Edman 1975)). About three days after a blood meal, the mosquito seeks an exposed area of mangrove (or salt marsh) for oviposition; clutch size is ca. 100. The eggs are able to hatch in three days if inundated from rain or tide. Thus, these mosquitoes are commonly referred to as floodwater mosquitoes. The newly hatched mosquito larvae, feeding on a rich supply of microorganisms and organic matter, complete their development (four larval instars) within 4-6 days then they pupate. The pupal stage lasts 24-48 hrs before adult mosquitoes emerge synchronously and renew the cycle.

The key to mosquito production apparently lies in the hydrological condition of the oviposition habitat. Repeated, relatively brief inundations of egg-bearing mangrove soil hatch eggs that produce more mosquitoes that lay more eggs, etc. This positive feedback cycle (Roberts et al. 1983a) is probably responsible for the dramatic population growth characteristic of the species. Obviously, mosquito production is proportional to the recycling rate of this cycle; rainy weather should produce more mosquitoes while dry weather should reduce production. However, prolonged and extensive flooding of mangrove basins appears to

decrease mosquito production (Ritchie 1984). Flooding can eliminate oviposition sites and often results in high populations of fish and aquatic insects that feed on mosquito larvae (Gilmore 1984, Provost 1977). Impoundment of mosquito-producing marshes has successfully diminished Ae. taeniorhynchus production (Clements and Rogers 1964, Provost 1977) and has generated considerable research on the relationships of marsh hydrology to estuarine ecology (Montague et al. 1985, Rey et al. 1986) and mosquito production (LaSalle and Knight 1974, Shisler et al. 1979). Unfortunately, little has been published on the relationship of hydrology to mosquito production in natural mangrove habitats.

Research on these relationships is essential to southwest Florida since this area is largely devoid of man-made mosquito control impoundments. Perhaps the rationale used to control mosquitoes in man-made impoundments can be used to predict mosquito populations in natural mangrove areas.

The objective of this project was to test the validity of this general hypothesis by designing a computer model (Montague et al. 1982, Roberts et al. 1983) to simulate the mangrove basin hydrology-saltmarsh mosquito system.

An intensive study format was used to test this hypothesis. The hydrology and Ae. taeniorhynchus production

were monitored carefully at two sites on Marco Island, FL from April 1985 to August 1987. These sites were chosen because they were accessible, historically produced large mosquito broods despite being subject to mosquito control and, most importantly, were ecologically and hydrologically diverse. One site, dominated by red mangrove, was almost exclusively flooded by rain and persistently produced salt marsh mosquitoes. The other site was dominated by black mangrove, was frequently flooded by tides and produced mosquitoes erratically. This study identified the key parameters needed to develop a predictive model of mosquito production for a diverse range of habitats. Ultimately, this study will provide the background to develop a network of hydrology-mosquito simulation models for mosquito surveillance and research.

The dissertation is organized into four general sections comprising nine chapters. The first (Ch. 1) introduces the dissertation topic. The second section describes the largest portion of the work involved collecting data from the field and in the laboratory to construct a mosquito simulation model. The egg stage received the greatest attention because little is known about its natural history in mangroves. Chapter 2 discusses methods developed to sample eggs in the field and the lab. Chapter 3 describes the distribution of eggs in a mangrove swamp. Chapter 4 details aspects of mosquito egg flushing (passive disper-

sal), a novel concept for Ae. taeniorhynchus. Chapter 5 describes natural mortality and suspected predators of salt marsh mosquito eggs. The next section (Ch. 6-8) detail construction and validation of the mosquito and hydrology models. The final section (Ch. 9) discusses the implications of this research and proposes future studies.

## CHAPTER 2
## THE DISTRIBUTION OF AEDES TAENIORHYNCHUS EGGS IN A SOUTHWEST FLORIDA MANGROVE FOREST

### Introduction

In order to verify a model simulating population dynamics, model predictions must be compared to field populations (Montague et al. 1982). The egg of Aedes taeniorhynchus is the best stage for model verification because absolute population estimates can be obtained by sampling. However, development of a sampling program requires knowledge about an animal's habitat preferences and the spatial distribution of the animal within different habitats (Southwood 1978). While it is known that black mangrove (Avicennia germinans Linne) basin forests are the typical oviposition site for Ae. taeniorhynchus in south Florida (Provost 1977), little is known about the egg distribution within this habitat. The objectives of this study are to describe qualitatively and quantitatively the distribution of Ae. taeniorhynchus eggs within a southwest Florida mangrove forest.

Qualitatively, floodwater mosquitoes appear to exhibit some common ovipositional behavior. Laboratory studies indicate that several species oviposit preferentially in response to substrate moisture (Knight and Baker 1962, Meek and Williams 1986, Russo 1978). Field studies support

this; eggs are frequently distributed in a horizontal band that maps the preferred soil moisture zone (Fallis and Snow 1983, Horsfall et al. 1973, Lefkovitch and Brust 1968, Novak 1981, Olson and Meek 1977). Thick detrital cover that limits soil desiccation has been associated with high egg populations (Horsfall et al. 1973, Russo 1978). Ultimately, specific plant-mosquito egg associations have been found that serve as an index of high egg populations. (Dale et al. 1986, Horsfall 1963, Scotton and Axtell 1979) This study will establish whether these characteristics are found in Ae. taeniorhynchus in south Florida.

The spatial distribution of individuals in a population provides vital information for the development of a sampling program (Southwood 1978). Several dispersion indices and mathematical models describe the spatial distribution and are incorporated in formulas calculating sample size (Southwood 1978, Taylor 1984). Typically, the distribution of floodwater mosquito eggs is contagious (Horsfall 1963, Lefkovitch and Brust 1968) and fits the negative binomial distribution model (Suggars et al. 1986). This implies that large numbers of samples are required for precise population estimates (Horsfall 1963, Southwood 1978). Additionally, the spatial distribution is used to choose sample sites, sample allocation and sampling pattern. Stratified and systematic sampling procedures require knowledge of an animal's distribution to maximize

sampling precision (i.e., the statistical validity of the population estimate). This chapter will describe the procedures used to obtain the spatial distribution information necessary to develop an efficient sampling program for the eggs of Ae. taeniorhynchus in a mangrove swamp (Ch. 3).

Additional studies were conducted to determine the ovipositional preferences of Ae. taeniorhynchus. A fortuitous series of events produced a natural experiment where oviposition could be correlated with several environmental parameters. Field and laboratory studies were conducted to validate the findings of this study.

## Materials and Methods

### Study Sites

The distribution of Ae. taeniorhynchus eggs was studied from 1984 to 1987 at two sites known to produce large populations of Ae. taeniorhynchus. Both sites are located on Marco Island, Florida, a barrier island in western Collier Co. The first site, Dogwood (named for an adjacent road), is a naturally impounded red mangrove (Rhizophora mangle Linne) forest covering ca. 0.7 ha (1.7 acres). In November 1984, a 37 X 49 m (120 X 160 ft) grid was marked and 109 elevations measured with a level and transit. These measurements were used to produce a topographic map of the grid using a mapping software package

(Surfer® (Golden Software, Inc. 1987); produced by Golden Software, Golden, CO; see Ch. 8 for details and topographic map).

A plot of the surface contours of Dogwood (Fig. 2-1) demonstrates the complex topography created by the red mangrove trees. A steep embankment (upper left) drops into a basin floor dominated by undulating hummocks created by the prop roots of red mangrove trees. This area is characterized by a thick carpet of red mangrove detritus overlying an extensive layer of peat; this substrate, representing the lowest elevations at Dogwood, is termed lowland. The lowland area then grades into a gently sloping area (right margin of Fig. 2-1) where black mangroves intersperse with red mangroves. This zone then grades into tropical hammock vegetation (representing the mean high water line) featuring rubber vine (Rhabdadenia biflora (Jacq.) Muell.-Arg.), golden leather fern (Acrostichum aureum L.), sable palm (Sabal palmetto (Walt.) Lodd. ex Schultes), sea grape (Coccoloba uvifera (L.) L.), southern fox grape (Vitis munsoniana Simpson) and poison ivy (Toxicodendron radicans (L.)). This soil, termed upland, has less detritus, is more compact and has a higher concentration of sand than lowland soil. The sand is probably carried by runoff from adjacent relict dunes during heavy rain.

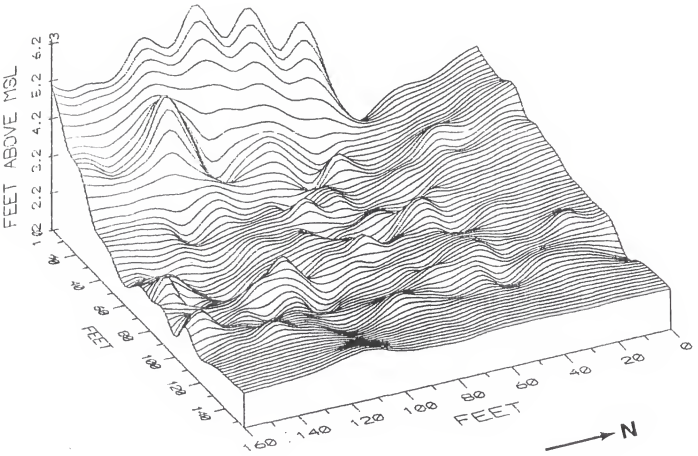



Figure 2-1. A surface contour plot (generated by Surfer®) of elevations (in ft above mean sea level) in a 37 X 49 m (120 X 160 ft) grid located in a red mangrove forest (Dogwood). The right hand edge of the plot faces north.

The other study site, April (also named for a nearby road) is dominated by black mangroves mixed with scattered stands of red mangrove. The substrate ranges from a firm sandy soil with a thin detrital covering of black mangrove leaves to a thick red mangrove detrital peat similar to Dogwood. Higher elevations feature a thin growth of Batis maritima L. April is topographically simple; it features a gentle sloping contour and lacks the hummocks and elevational range of Dogwood.

The two sites differ hydrologically. Maximum depth of the central pool was 0.49 m (1.6 ft) for Dogwood but only 0.25 m (0.8 ft) for April. Thus, April dried both more quickly and more frequently than Dogwood. Both sites were usually dry by late spring before becoming fully inundated by summer rains. Tidal flooding patterns also were dissimilar. A high berm protected Dogwood from tidal inundation except during unusual storm events. April had a shallow berm which allowed tidal flooding several times each year.

Habitat Associations

The distribution of Ae. taeniorhynchus eggs was studied using the technique of Bidlingmayer and Schoof 1956). Upland and lowland sites featuring a full elevational range (such as red mangrove hummocks) were sampled systematically (Cochran 1963); sample locations

were defined by transects with rows and columns parallel and perpendicular to elevational contours, respectively. Rows were initiated at elevations slightly above the water table; row elevations were determined from the topographic map. A 10-cm (4 in) diameter golf hole corer was used to take samples; from 50 to 100 samples/site were collected per day. Each sample was placed in a 500 ml plastic cup (diameter of 10-cm), labeled (location, date) and incubated at ca. 28 °C for at least three days to allow recently laid eggs to mature and/or break "diapause" in winter (Moore and Bickley 1966, Nayar 1985). Samples were then flooded with a dilute yeast solution and after 24 hr the larvae were counted. Concurrent studies of percent hatch and observations of larval survival suggest that approximately 90% of the hatchable eggs were tallied (assuming no predation and induced mortality).

Emergent red mangrove prop roots and black mangrove pneumatophores are potential oviposition sites, especially when the forest floor is flooded. Prop roots were sampled for eggs by cutting bark strips (ca. 80 X 25 mm) at the waterline with a pocketknife. Pneumatophores were clipped below the waterline with tinsnips. Eggs were removed by agitating the root section in water and collected in a 150-um sieve. The sieved material was then bleached for 5 min (see Ch. 3) before counting eggs.

Spatial Distribution of Eggs

The spatial distribution and its relationship to elevation were investigated using the Dogwood samples. Spatial distribution was described by the dispersion index k (Southwood 1978) and the parameter b in Taylor's Power Law (Taylor 1984). Ten egg populations from Dogwood (4 lowland, 6 upland) were tested with $X^2$ for goodness of fit (fit is acceptable if $P > 0.10$) to a negative binomial distribution model (Elliott 1971). The relationship between egg density and elevation was investigated by regressing cumulative egg frequency and transformed cumulative egg frequency against elevation for three different hydrologic conditions (dry, partially flooded and completely flooded forest). The transformation, $\ln(f/1-f)$, where f is the proportion of eggs within a specified elvational range, was used to linearize the functional relationship between elevation and cumulative egg frequency (J. Allen, personal communication).

Oviposition Preferences: Field Studies

Preliminary data suggested that thick detrital substrates were associated with high egg populations. Hypothetically, Ae. taeniorhynchus oviposition may be limited to red mangrove detritus in black mangrove forests since thick detritus is concentrated under scattered stands of red mangrove. Leaf litter is relatively scarce under

monospecific black mangrove stands at April. A snail may be responsible; large populations of the pulmonate snail, Melampus coffeus Linne have been observed grazing on leaf litter in this area. Experiments were undertaken to compare the decomposition rates of red and black mangrove leaves at April.

Following a large oviposition event, populations of salt marsh mosquito eggs, M. coffeus, and red and black mangrove leaf litter were compared in an April transect. One hundred 10-cm diameter cores were collected on 28 July 1987 in an area (60 X 9 m) transecting stands of black mangrove and mixed red and black mangrove. Egg populations were determined by flooding, snails were counted and red and black mangrove litter densities estimated by counting leaves > 50 % intact in each sod. Depth to water table was measured for each core hole. These data probably represent oviposition for a short period because the site had only dried recently (25 July 1987) after prolonged flooding (basin nearly inundated since 26 June 1987). No movement of eggs from flushing should have occurred because no rain had occurred since 25 July 1987.

The data were analyzed in two ways. The correlation between the five parameters (eggs transformed by log(X + 1)) was calculated and stepwise linear regression (forward option; Freund and Littell 1986) was used to find the combination of parameters that accounted for the most

variability in the transformed number of eggs/sample. Surfer® (Golden Software, Inc. 1985) was used to construct surface contour plots so the distribution of the snails, litter and mosquito eggs in the grid could be compared visually. Kriging was used to estimate grid points and cubic splining was used to smooth the surface contour (default settings were used for both procedures).

The decomposition rates of red and black mangrove leaves were compared in the field to see if differences could account for the accumulation of red mangrove litter at April. Similarly sized, succulent red and black mangrove leaves were collected from the forest floor and secured individually side-by-side to the soil with a toothpick. In trial 1, 5 sites with 4 leaf pairs/site were set in the field from 22-25 July 1987. In trial 2, 4 sites with 6 leaf pairs each were placed in the field from 28 July to 2 Aug. 1987. A control leaf, placed in a fiberglass screen pouch (0.15 cm openings) to prevent direct access by snails, was placed at each test site.

Initially, the weight loss/leaf was used to quantify leaf decomposition. Unfortunately, changes in leaf water content confounded results. Therefore, leaf damage was scaled. Field observations indicated that skeletonization was initiated on the leaf surface (surface skeletonization), spread over the leaf then deepened (complete

skeletonization) until nothing but the thickest leaf veins remained. This feeding pattern has been documented for M. coffeus on red mangrove leaves (Lopez et al. 1977). The leaf damage scale was as follows: 0 (no damage), 1 (< 25% surface skeletonized), 2 (25 to 50% surface skeletonized), 3 (50 to 100% surface skeletonized), 4 (100% surface skeletonized with some complete skeletonization). The damage to red and black mangrove leaves was compared using a nonparametric one-way analysis of variance procedure (Schlotzhauer and Littell 1987).

Oviposition Preferences: Laboratory Studies

Oviposition preference for red mangrove litter was also tested in the lab. Eight 500 ml plastic cups, four from each treatment, were arranged in a square (3 cups/side, position chosen randomly) in a 0.5 $m^3$ cage containing gravid field-collected Ae. taeniorhynchus. After 24 hr, the sods were removed and the eggs were sieved, bleached and counted; the experiment was replicated three times. Treatment differences between percentage of total eggs for a cup (eggs in a cup/sum of eggs in 8 cups) were examined with the Mann-Whitney U test (Schlotzhauer and Littell 1987).

The vertical distribution of eggs in red mangrove detritus was examined by exposing similarly caged mosquitoes to upland and lowland sods collected at Dogwood. To

minimize resident eggs that might bias the data, only sods exhibiting no eggs when flooded were used. Leaves were removed by layer and agitated in water for 30 seconds to remove eggs. The water was sieved (150 um) and bleached to facilitate egg counting.

## Results

### Habitat Associations

Sampling of Ae. taeniorhynchus eggs at Dogwood indicated that oviposition occurs almost exclusively on the detritus-rich soil (Table 2-1; a summary of descriptive statistics for all egg surveys is presented in Appendix A). Detritus-free mud, pneumatophores and red mangrove prop roots contained essentially no eggs. Highest egg densities were found in the thick red mangrove detritus of the lowland stratum. Highest upland egg densities occurred when lowland areas were flooded.

Table 2-1. Aedes taeniorhynchus eggs collected from different substrates in a mangrove forest (Dogwood)

| Stratum, substrate | sampling sessions, total no. samples | Eggs/sample (mean, SD) |
|---|---|---|
| pool bottom, mud | 1, 30 | 0, 0 |
| lowland, thick detritus | 7, 492 | 25.2, 59.0 |
| upland, thin detritus | 9, 646 | 3.6, 7.1 |
| red mang., prop roots | 3, 276 | 0, 0.1 |
| black mang., pneumatophores | 2, 200 | 0, 0 |

The distribution of Ae. taeniorhynchus eggs indicates that most eggs are located within 0.5 ft (0.15 m) of the water level (Fig. 2-2). The functional relationship appears similar for each regime, although steeper with higher water levels. The log-transformed egg data provided higher $r^2$ values than the nontransformed egg data when regressed against elevation for dry ($r^2$ = 0.97 vs. 0.78) and completely flooded ($r^2$ = 0.93 vs. 0.77) conditions. Values of $r^2$ were similar during partially flooded conditions (0.88 vs. 0.90 for the transformed and nontransformed data, respectively). Eggs were concentrated in a horizontal band at lowland sites (Fig. 2-3) while no distinct pattern was noticed with upland populations (Fig. 2-4).

## Spatial Distribution

Aedes taeniorhynchus eggs are very clumped in distribution. The dispersion parameter k was 0.14 $\pm$ 0.09 (mean $\pm$ SD) for 8 upland populations and 0.25 $\pm$ 0.22 for 7 lowland populations. These low values ($k < 1$) are indicative of a contagious distribution (Southwood 1978). Calculation of Taylor's Power Law (Taylor 1984) produced a b parameter of 1.662 (Fig. 2-5), also indicative of a highly clumped distribution. Clumping was also evidenced by the fit of egg sampling data to the negative binomial; this model provided a good fit for 9/10 populations tested. The contagious distribution of egg populations is perhaps

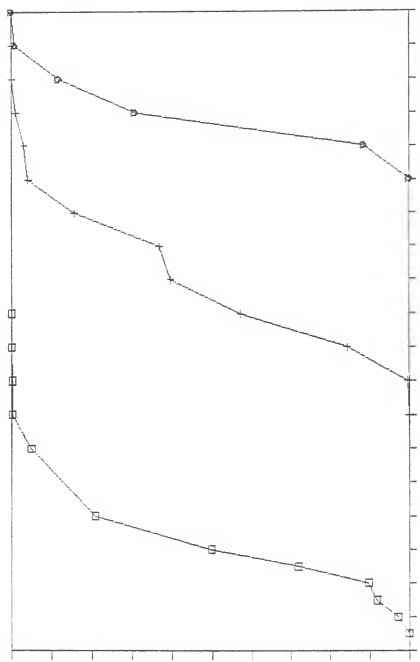



Figure 2-2. A plot of the cumulative egg distribution at Dogwood for three hydrologic regimes.

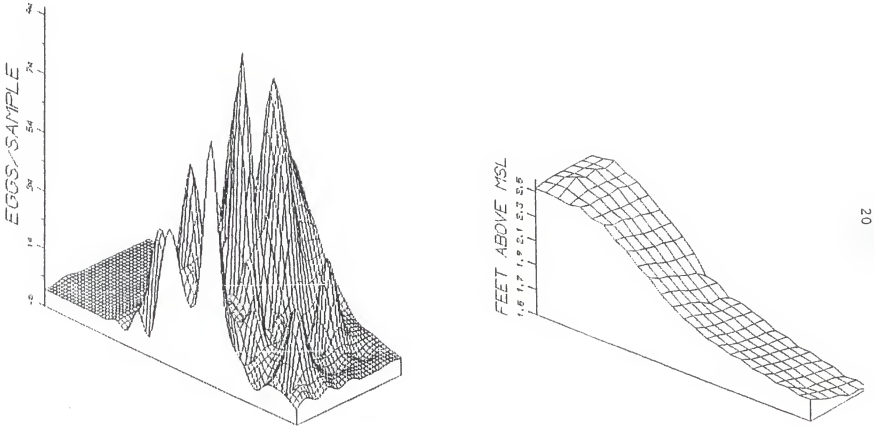



Figure 2-3. A surface contour plot of an Aedes taeniorhynchus egg population (left) and elevations (right) at a lowland site at Dogwoood (sampled 30 May 1986). The sample grid (4 X 1.6 m) was located on a red mangrove hummock.

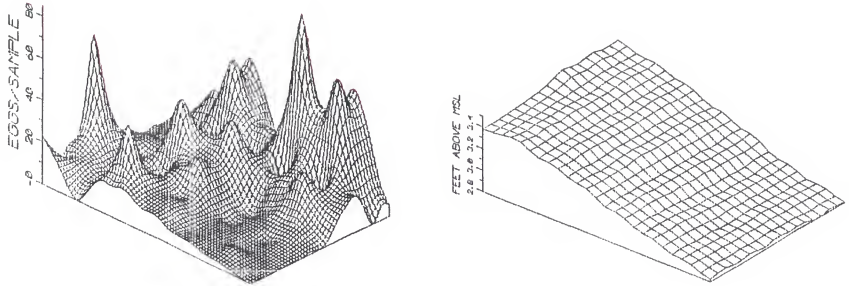



Figure 2-4. A surface contour plot of an Aedes taeniorhynchus egg population (left) and elevations (right) at an upland site at Dogwood (sampled 14 July 1986; grid size 5 X 3.6 m).

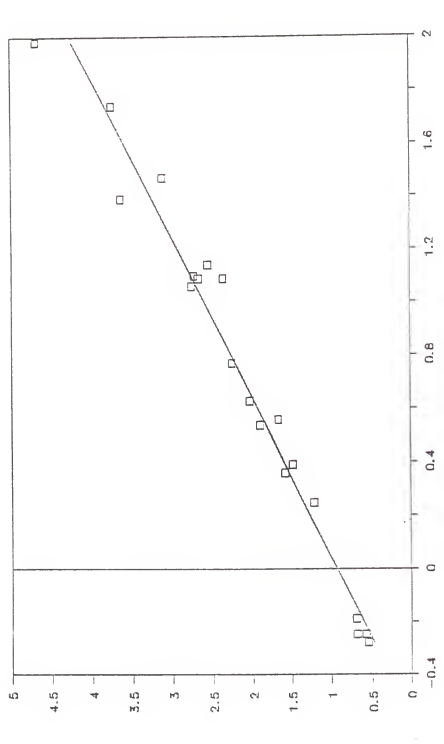



Figure 2-5. A plot of log mean vs log variance for 20 Aedes taeniorhynchus egg populations sampled at Dogwood. The regression line, $Y = 0.32 + 1.66X$ where 1.66 represents b in Taylor's Power Law), is shown.

comprehended best by the surface contour plots of egg populations (Figs. 2-3 and 2-4).

Oviposition Preferences: Field Studies

The relationship between Ae. taeniorhynchus populations and mangrove litter is dynamic. The correlations between transformed (log(X+1)) number of eggs per sample, depth of the water table, number of M. coffeus per sample, and the number of red and black mangrove leaves per sample are summarized in Table 2-2; raw data is provided in Appendix B. The transformed number of mosquito eggs per sample was positively correlated to the number of red mangrove and black mangrove leaves per sample. The number of red and black mangrove leaves per sample was negatively correlated with the depth to the water table and with the number of snails per sample, suggesting that exposed leaf litter may be reduced by grazing M. coffeus. The negative correlation between the number of snails per sample and depth to water table indicates that snail populations were higher in drier areas. The relationships can be visualized in the surface contour plots of the variables (Figs. 2-6 and 2-7).

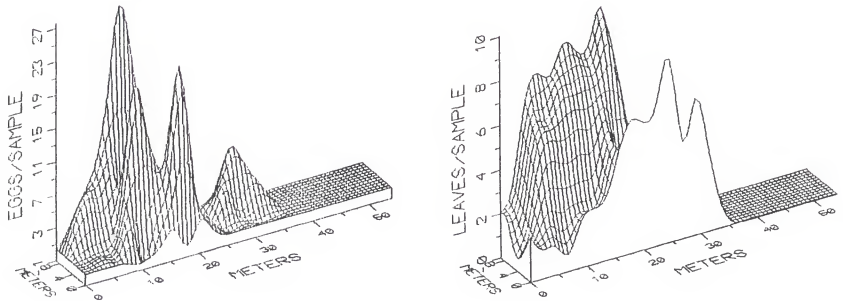



Figure 2-6. Surface contour plots for Aedes taeniorhynchus eggs (left) and red mangrove leaves > 50% intact (right) for the April grid (samples collected on 28 July 1987).

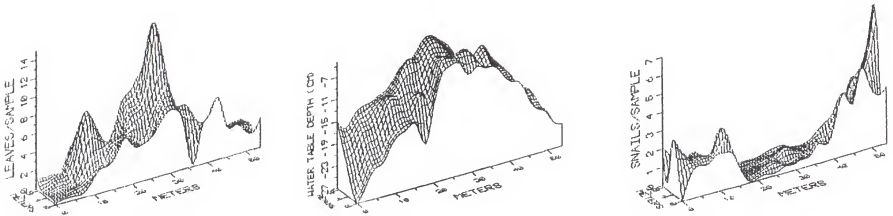



Figure 2-7. Surface contour plots for the number of black mangrove leaves > 50% intact/sample, the depth to the water table and the number of Melampus coffeus/sample, respectively, at the April grid (sampled on 28 July 1987).

Table 3-2. Correlation coefficient for several environmental variables collected at April. Data consisted of 100 samples collected systematically from a 60 X 9 m grid; values are r for the parameters at the intersecting row and column. RED and BLACK refer to number of red and black mangrove leaves per sample, WT to the depth to the water table, and SNAIL and EGGS to number of salt marsh mosquito eggs and M. coffeus per sample, respectively.

| | RED | BLACK | SNAIL | WT |
|---|---|---|---|---|
| EGGS | +0.40 | +0.11 | -0.15 | -0.11 |
| RED | - | +0.04 | -0.24 | -0.20 |
| BLACK | +0.04 | - | -0.19 | -0.49 |
| SNAIL | -0.24 | -0.19 | - | +0.45 |

Further analysis indicated that there is a positive relationship between water level and the number of mosquito eggs. This analysis only involved samples positive for red mangrove litter because mosquito eggs were almost limited exclusively to this habitat. The correlation of the transformed number of mosquito eggs per sample to the depth to the water table depth was not significant. However, using a quadratic model (i.e., water table and water table $^2$), the $r^2$ increased from 0.009 to 0.140. While technically not a significant regression, the data suggest that oviposition is concentrated in a preferred soil moisture zone.

The results from the stepwise regression analysis indicate that the number of red mangrove leaves per sample is the only variable that accounted for a significant amount of the variability ($r^2 = 0.160$) in the transformed number of eggs per sample. Addition of the number of black mangrove leaves and the depth to the water table resulted in a $r^2$ of 0.168 and 0.169, respectively. The number of snails per sample did not contribute significantly to the model.

The results of the leaf exposure tests support the hypothesis that red and black mangrove leaves break down at signficantly different rates. For trial 1, the mean leaf damage score was 0.43 and 1.29 for red and black mangrove leaves, respectively; a significant difference ($P = 0.02$). For trial 2, the mean leaf damage score was 0.25 and 1.83 for red and black mangroves leaves, respectively; a highly significant difference ($P = 0.0005$). No damage was noticed on the control leaves, suggesting that leaf damage was caused by an animal that could not penetrate the screen. Observations of numerous M. coffeus on the test leaves suggest that this snail may be responsible for the damage. Mook (1985) established that M. coffeus consumes red mangrove leaves readily. The data also suggest that this snail may prefer black mangrove leaves to red mangrove leaves.

Oviposition Preferences: Laboratory Studies

Laboratory studies confirmed an oviposition preference for red over black mangrove substrate. The mean percentage ($\pm$ SD) of eggs per sod for each experiment was 8.15 $\pm$ 4.4 % for black mangrove substrate vs 16.8 $\pm$ 6.5 % for red mangrove substrate, a highly significant difference ($P < 0.01$).

Aedes taeniorhynchus apparently oviposit well below the surface litter layer, especially for lowland soil. The data (Fig. 2-8) indicate that most eggs were deposited deep within the peat. For upland soil, a majority (mean = 58.3%, SD = 35.2%) of eggs were laid on the soil underlying the single layer of detritus.

## Discussion

The data exhibit many of the documented characteristics associated with the distribution of floodwater mosquito eggs. Preferred oviposition sites within a mangrove basin forest were established, eliminating substrates such as red mangrove prop roots and black mangrove pneumatophores and detritus-free pool bottoms from consideration as sampling sites for salt marsh mosquito eggs. A strong vegetation - mosquito egg association was established for red mangrove leaf litter. Aedes taeniorhynchus eggs were distributed in a horizontal band, presumedly in response to soil moisture concentrations. The spatial

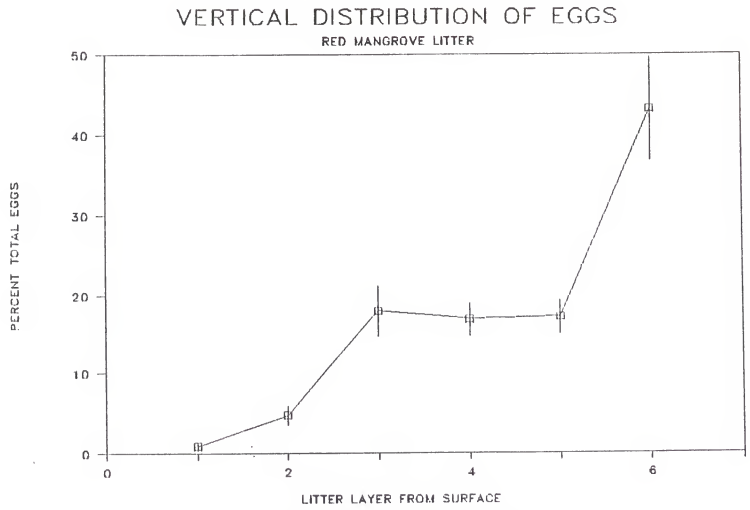



Figure 2-8. A plot of the distribution of eggs through successive layers of red mangrove detritus (lowland soil). The values are mean ± SE proportion of eggs per layer for 10 replications.

distribution of eggs was highly clumped, indicating that a large number of samples is necessary in order to provide precise estimates of the population.

However, these results can be used to minimize the number of samples needed for a fixed precision level. Obviously, sampling should only be conducted at areas that are likely to contain Ae. taeniorhynchus eggs. The identification of distinct egg populations for upland and lowland soils in Dogwood can be used to develop an optimal sample allocation program (O'Neil and Stimac 1985) based on stratified sampling (Cochran 1963). The graded egg distribution for lowland soils can be sampled systematically with a potential gain in sampling precision (Cochran 1963). The functional relationship of egg density to elevation can be used to partition oviposition in simulation models. Chapter 3 will examine the application of these ideas in the development of a sampling program for the eggs of Ae. taeniorhynchus at Dogwood.

The oviposition preference studies indicated that several dynamic mechanisms appear responsible for the distribution of salt marsh mosquito eggs in a mangrove forest. Mixed mangrove forests apparently have quite distinct substrates within the same basin. Aedes taeniorhynchus oviposition is apparently restricted to thick detrital substrates typically found under red mangrove trees. It is interesting than Provost (1977) suggested that black man-

grove basin forests represent the primary domain of salt marsh mosquito production in south Florida. These data suggest that red mangrove litter is a limiting agent for salt marsh mosquito oviposition within a basin forest. The accumulation of thick litter overlaying mangrove peat apparently creates an attractive oviposition substrate that may protect eggs from desiccation and provide an abundant food supply for larvae.

This mechanism would explain why salt marsh mosquito eggs were not found in the monospecific black mangrove stand at April. The forest floor lacked the extensive surface litter and peat associated with the mixed forest. This could be caused by lower litter fall rates (Twilley et al. 1986) and by faster leaf decomposition rates. The results of the leaf decomposition study indicate that black mangrove leaves break down at a faster rate than do red mangrove leaves. They may be grazed preferentially or they may be less resistant to skeletonization; black mangrove leaves lack the thick, waxy cuticle of red mangrove leaves. The pulmonate snail, M. coffeus appears to be a significant factor in the rapid decomposition of black mangrove litter. Perhaps this animal could be used control leaf litter buildup in black mangrove basin forests, effectively reducing salt marsh mosquito oviposition. However, additional black mangrove basin forests need to be studied before generalizations can be made. It would be

interesting to study the litter and mosquito egg dynamics in a black mangrove forest devoid of M. coffeus.

# CHAPTER 3
# METHODS FOR SAMPLING AEDES TAENIORHYNCHUS EGGS IN THE FIELD AND LABORATORY

## Introduction

The egg of aedine mosquitoes is often targeted for sampling because it is the only lifestage with "concentrated and stable" populations (Horsfall 1956). A great deal of information regarding the distribution and habitat associations of floodwater and salt marsh mosquito eggs has been generated (Horsfall et al 1973, Scotton and Axtell 1979, Meek and Olson 1976, Suggars et al. 1986). Because the objective of these studies was to define relative populations in different habitats, a statistically rigorous sampling protocol was not used. However, the development of a simulation model for population prediction necessitates a comprehensive sampling program that will provide accurate population estimates.

Studies on the occurrence and distribution of Ae. taeniorhynchus eggs (Ch. 2) provide data for development of such a sampling program. Egg populations had a contagious distribution characterized by a low mean to variance ratio (Ch. 2). Populations with contagious distributions are often difficult to sample (Southwood 1978); large numbers of samples are required and many areas are nonproductive. Fortunately, habitats that consistently contained

eggs were identified; impounded red mangrove and mixed red and black mangrove forests were found to contain Ae. taeniorhynchus eggs throughout the year. The statistical parameters used in sample size and allocation, namely the dispersion index k and the sample mean and variance, were calculated. For the impounded red mangrove site (Dogwood), egg populations from lowland and upland areas were found to have distinct mean and variance (Ch. 2) and could serve as strata in an optimal sampling program (O'Neil and Stimac 1985). The objective of optimal sample allocation is to maximize sampling efficiency by minimizing sampling costs and sample variance. Additionally, egg densities appeared contoured relative to elevation. This is a situation in which systematic sampling can be used in order to minimize sample variance and maximize sampling precision (i.e., minimize the width of a given confidence interval (Cochran 1963)).

The objective of this study was to design a sampling program for the eggs of Ae. taeniorhynchus at Dogwood. The sampling precision of systematic sampling (Cochran 1963) was examined and used to provide data with which to design an optimal sampling program (O'Neil and Stimac 1985). Such a program should be applicable to other sites and aedine mosquitoes with similar oviposition habitats.

Sampling of large numbers of mosquito eggs in laboratory studies of the hatching and flushing of Ae.

taeniorhynchus eggs necessitated development of a procedure to process rapidly and count accurately the mosquito eggs present in soil. The sieving and flotation method developed by Horsfall (1956) satisfactorily separated eggs from most soil but still required extensive microscopy to recover eggs. Preliminary observations indicated that a 5% sodium hypochlorite solution (100% household bleach), commonly employed to clear the mosquito egg chorion (Morterson 1950, Trpis 1970), could be used to selectively bleach the background detritus so that mosquito eggs could be counted with the unaided eye.

Dogwood soil contained high concentrations of relict Ae. taeniorhynchus eggshells that superficially resembled recently-hatched eggshells. Sodium hypochlorite might be used to bleach relict eggshells in order to distinguish them from new eggs. Finally, sodium hypochlorite might be used to examine fecal samples for the presence of egg chorion.

## Materials and Methods

### Egg Sampling Program for the Field

Data collected from 1984 to 1986 and used in development of the Ae. taeniorhynchus sampling program were collected at the Dogwood site (described in Ch. 2) on Marco Island, Florida. Samples were taken systematically (Cochran 1963) as described in Ch. 2. Data analyses involved calculation of sample size (i.e., the number of samples needed to esti-

mate the population mean for a given precision level) and variance for the strata identified at Dogwood (i.e., lowland and upland substrates). These data were used to develop an optimal sampling program (O'Neil and Stimac 1985) for the eggs of Ae. taeniorhynchus in similar habitats.

Estimated sample size was calculated for each stratum. Because the spatial distribution of eggs was found to fit the negative binomial distribution (i.e., distribution of eggs was clumped; see Ch. 2), the following sample size formula (Eq. 3-1; Rojas 1964) for populations described by the negative binomial was used:

$$n = \frac{(1/\overline{X} + 1/k)}{E^2} \qquad (3\text{-}1)$$

where $n$ = calculated sample size (i.e., number);

$\overline{X}$ = sample mean;

$k$ = the dispersion index and

$E$ = the desired precision level.

Sample size over a range of precision levels (e.g. a precision level of 0.10 produces a 95% confidence interval with a half width ca. equal to the sample mean X 0.10 X 2) was calculated from lowland and upland egg populations.

Calculation of sample S (variance) for lowland and upland strata was done using the following modification (Eq. 3-3) of Cochran's (1963) formula for systematic

samples (Eq. 3-2):

$$V_{ys} = \frac{S^2}{n}(1 + (n-1)\rho) \qquad (3\text{-}2)$$

where $V_{ys}$ = the variance for samples collected systematically;

$S^2$ = the sample variance;

$n$ = number of samples and

$\rho$ = rho, the correlation (sign ignored) between adjacent systematic samples.

Equation 3-2 can be simplified (Eq. 3-3) for large sample numbers as follows:

$$V_{ys} = S^2(1+\rho) \qquad (3\text{-}3)$$

This is possible because n-1 and n are present in the numerator and denominator of Eq. 3-2, respectively, and, thus, effectively reduce to 1 as n gets large. Thus, positive and negative values of rho increase and reduce Vys, respectively.

Rho was calculated in two ways. First, rho was calculated for samples in the same row, thereby "sampling" parallel to elevation contour; this is termed parallel systematic sampling. Second, rho was calculated between samples in the same column, thereby sampling perpendicular to elevation contour; this is referred to as perpendicular systematic sampling. Sample allocation was calculated using a modification (Eqs. 3-5 and 3-6) of the following

sample allocation formula (Eq. 3-4) from O'Neil and Stimac (1985):

$$n_h = \frac{N_h S_h}{\sqrt{C_h}} \Bigg/ \left[ \sum_{n=1}^{L} \frac{N_h S_h}{\sqrt{C_h}} n \right] \qquad (3\text{-}4)$$

where n = sample size;

h = stratum;

N = available area;

S = sample standard deviation and

C = sample cost.

The available area per stratum, $N_h$ was assumed to be equal because exposed area of any particular stratum is difficult to quantify a priori due to changes in the water table. Therefore, $N_h$ was dropped from the formula (Eq. 3-5);

$$OSI_h = \frac{S_h}{\sqrt{C_h}} \Bigg/ \left[ \sum_{n=1}^{L} \frac{S_h}{\sqrt{C_h}} \right] \qquad (3\text{-}5)$$

where OSI = the optimal sample allocation index, and other parameters as in Eq. 3-4.

This index was used to calculate $n_h$ as shown in Eq. 3-6:

$$n_h = OSI_h \times n \qquad (3\text{-}6).$$

The sample variance, $S_h$, was calculated from the mean of five and seven populations sampled for lowland and upland strata, respectively. Sampling and processing time was the only cost difference between samples. Thus, $C_h$, the cost to sample a stratum, was measured in time units (seconds).

Sample cost per stratum measures the mean sampling time for lowland and upland strata. Sampling cost consists of the time spent taking samples, flooding samples, counting larvae and cleaning the laboratory. Time was estimated from a typical sample survey (100 samples) and is expressed in seconds per sample. Experience indicates that sampling, flooding and cleanup times are equivalent for each stratum. However, because the mean number of larvae/sample was considerably higher (22.9 vs 4.5) for lowland than upland sites, the lowland counting time (i.e., cost) should also be higher. The functional relationship between number of larvae and counting time was estimated by linear regression. The time required to count known numbers of larvae placed in flooded, mangrove sod-filled plastic deli dishes (10-cm diameter, 8.5-cm height) was measured and regressed against larval number. This model was used to estimate counting time for the mean number of eggs/stratum. A final optimal sampling index (OSI) was calculated using Eq. 3-5. This value was multiplied by the overall sample size to obtain the optimal sample allocation for a stratum (Eq. 3-6).

Sampling Eggs in the Laboratory

The clearing time of eggs and new and relict eggshells in 2.5% sodium hypoclorite (50% commercial bleach) was estimated. Eggs collected from wild Ae. taeniorhynchus females were incubated at room temperature for one week before testing. New egghells were collected after hatching some of these eggs. Relict eggshells were collected from Dog-wood soil that had been passed through a 150-um screen. A group of 10 eggs or 10 eggshells was placed in a watch glass and ca. 1 ml of 2.5% sodium hypochlorite added. The number of eggs or eggshells with any remaining chorionic pigment was tallied after a 5-min exposure. The experiment was replicated 10 times and the mean number of uncleared eggs or eggshells was compared for significant differences with multiple 't' tests. (Schlotzhauer and Littell 1987). On four occasions, the time for all 10 eggs to clear was measured.

A study was conducted to see if 5% sodium hypochlorite could be used to verify if an animal had ingested Ae. taeniorhynchus eggs. Several hundred Ae. taeniorhynchus eggs and a small piece of Purina cricket chow were placed in a 100-ml plastic container containing three adult Gryllus sp. After 48 hr, cricket feces were collected and examined microscopically for mosquito egg fragments. Egg fragments were identified by a dark metallic sheen and a sculptured surface (Craig 1955). A drop of 5% sodium

hypochlorite was added; bleaching was facilitated by teasing the feces apart with forceps.

## Results

### Systematic Sampling of Natural Egg Populations

Calculated sample size for Ae. taeniorhynchus eggs was very high and increased dramatically as precision levels dropped (Fig. 3-1). This reflects the low values of k due to the extremely clumped distribution of eggs. Southwood (1978) suggests that precision levels of 0.05 to 0.10 are desired when sampling to estimate populations. Thus, sample sizes exceeding 1,000 would be necessary in order to sample with precision levels less than 0.10.

While choice of sample size should reflect acceptable precision levels, it is ultimately determined by available resources and population stability. Unfortunately, precision levels below 0.20 were not obtainable with the resources of this study. Personal experience indicates that two people can reasonably take ca. 100 samples/day in mangrove forests for Ae. taeniorhynchus eggs. Perhaps precision could be increased by sampling over a period of several days. However, sampling within 24 hours is imperative because an intervening rain or flooding tide could hatch eggs and substantially alter the population. For example, an upland site sampled on 17 and 18 July 1986 had respective

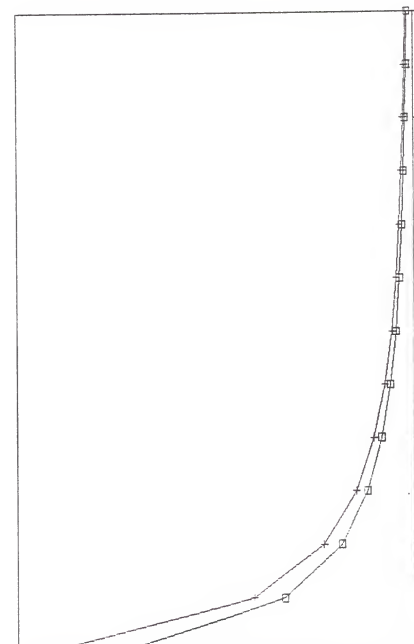



Figure 3-1. A plot of the calculated sample size using the formula of Rojas (1964) for lowland and upland Aedes taeniorhynchus egg populations at Dogwood.

means of 12.4 and 0.6 eggs/sod. A brief but heavy rainfall (0.63 in, 1.60 cm) apparently produced substantial egg flushing (see Ch. 4) that dramatically reduced the size of the egg population. While larger sample size would increase precision (from 0.24 to 0.20 with an increase from 100 to 150 samples), the small gain in precision is negated by the logistical problems discussed. Therefore, a sample size of 100 was selected for use in the optimal allocation formula despite a precision level of only 0.24 (Southwood (1978) recommends using a precision level of 0.10 if possible!).

Aedes taeniorhynchus eggs often were found concentrated in a band parallel to elevation contours, most noticeably for lowland sites (see Figs. 2-3 and 2-4, pp. 20-21). Banding of egg populations relative to elevation and soil moisture is common in floodwater mosquitoes (Fallis and Snow 1983, Horsfall et al. 1973, Lefkovitch and Brust 1967) and offers potential in maximizing precision in systematic sampling program. A systematic sampling scheme that reduces rho (correlation between adjacent systematic samples) would reduce variance and thus maximize precision. One would expect that by sampling parallel to elevation contours, rho would be positive while by sampling perpendicular to elevation contours rho would be negative. In Table 3-1 values of rho and resulting variance are shown for both sampling schemes for several egg populations

sampled from lowland and upland areas of Dogwood; S is shown since it is used in calculation of optimal sample allocation (Eq. 3-4).

Table 3-1. Values of rho and S calculated by systematic sampling parallel and perpendicular to elevation contour for lowland and upland sites at Dogwood.

| Stratum date | Rho | | S | |
|---|---|---|---|---|
| | Paral. | Perp. | Paral. | Perp. |
| I. Lowland | | | | |
| A. May | 0.20 | -0.04 | 25.1 | 22.5 |
| B. June | 0.11 | 0.01 | 187.3 | 178.6 |
| C. June | 0.0 | -0.04 | 22.0 | 21.2 |
| D. October | 0.07 | 0.01 | 13.9 | 13.5 |
| Mean values | 0.10 | -0.02 | 62.1 | 58.9 |
| II. Upland | | | | |
| A. June | -0.13 | 0.06 | 5.7 | 6.3 |
| B. July | 0.36 | 0.07 | 19.7 | 17.8 |
| C. July | 0.29 | -0.04 | 2.5 | 2.2 |
| D. August | 0.49 | 0.17 | 4.8 | 4.3 |
| E. August | -0.05 | 0.26 | 2.0 | 2.3 |
| F. October | -0.07 | -0.09 | 6.8 | 6.7 |
| Mean values | 0.15 | 0.07 | 6.9 | 6.6 |

Systematic sampling perpendicular to elevation contour did result in a slight decrease in S and increase in sampling precision over SRS at lowland sites at Dogwood. The mean precision index ($S^2$ simple random sampling / $S^2$ systematic sampling (Cochran 1963)) for lowland populations was 0.91 and 1.02 for parallel and perpendicular systematic sampling, respectively. However, parallel systematic sam-

pling did not consistently reduce the variance for upland egg populations; the respective mean precision index was 0.93 and 0.86 for parallel and perpendicular systematic sampling schemes. This probably reflects the lack of a distinctively banded egg distribution.

The impact of parallel and perpendicular systematic sampling on the calculated dispersion index, k (Southwood 1978) and sample size (Eq. 3-1; Rojas 1964) for lowland egg populations is shown in Table 3-2. While perpendicular systematic sampling does provide greater sampling precision than SRS and parallel systematic sampling, the improvement over SRS is slight. Since neither parallel nor perpendicular systematic sampling resulted in greater precision than SRS (note that the mean precision indexes are less than 1) for egg data from upland sites, no improvement in sample size was obtained.

Table 3-2. Mean impact of parallel and perpendicular systematic sampling on dispersion index (k) and parameters associated with sample size calculation (Rojas 1964) (data from four lowland Dogwood populations).

| Sampling Method: | simple random | systematic paral. | systematic perp. |
|---|---|---|---|
| Parameter | | | |
| k, dispersion index | 0.149 | 0.137 | 0.152 |
| E, desired precision when n = 100 | 0.260 | 0.270 | 0.257 |
| n, sample size when E = 0.25 | 108.2 | 117.3 | 105.7 |

Development of an Optimal Sample Allocation Procedure

Calculation of optimal sample allocation for Ae. taeniorhynchus eggs was conducted using lowland and upland areas at Dogwood as strata. Values of $S_h$ used were 61.3 and 8.8 for lowland and upland strata, respectively. Calculation of $C_h$, cost to sample a stratum, was estimated by the time spent taking samples, flooding samples, counting larvae and cleaning. For a typical day's sampling (100 samples), it takes ca. 5 hrs to take the samples (180 sec/sample), 1 hr to flood the samples (36 sec/sample) and 1 hr for laboratory cleanup (36 sec/sample). The cost in seconds to count larvae was determined by regressing larval number against counting time (Fig.3-2). The regression model for larval counting time vs number of larvae which

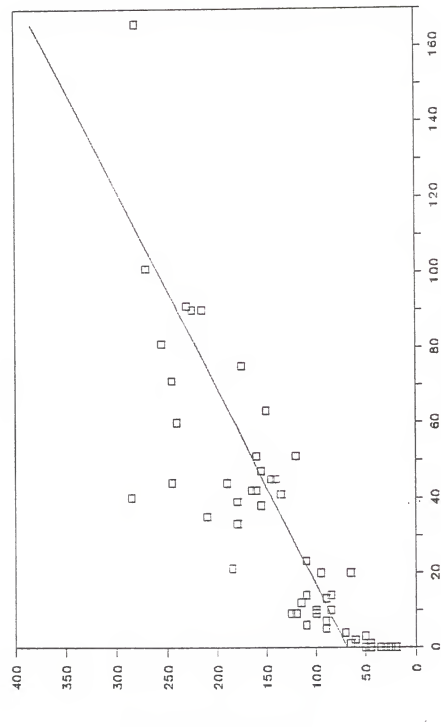



Figure 3-2. A plot of the number of larvae per sample and counting time. The regression line, Y = 68.4 + 1.90X, is shown.

was Counting Time = 68.4 + 1.90(number of larvae in a sample); n = 58, range = 0 - 166, $r^2$ = 0.71, slope significant at $P < 0.001$. The estimated larval counting time (based on 22.9 and 4.5 larvae/sample - see Table 3-1) was 111.9 and 77.0 sec/sample for lowland and upland strata, respectively. Thus, the respective values of $C_h$ were 367.6 and 330.5 for lowland and upland strata. The final parameter values used to calculate optimal sample allocation are listed in Table 3-3.

Table 3-3. Parameter values used in calculation of optimal sample allocation for sampling Aedes taeniorhynchus eggs. Strata cost is the per sample cost (in seconds) for each strata. Strata S is the standard deviation of the mean eggs per sample per stratum. OSI is the optimal sample index - the proportion of samples taken in each strata.

| Stratum | strata cost | strata S | OSI |
|---|---|---|---|
| Lowland | 363.9 | 61.3 | 0.87 |
| Upland | 329.0 | 8.8 | 0.13 |

The optimal sampling procedure indicates that 87% and 13% of the samples should be taken from lowland and upland sites, respectively. However, the sample size calculations using Rojas' (1964) formula indicate that slightly more samples should be taken from upland sites (Fig. 3-1) This paradoxical situation reflects the mathematical differences in the formulas. The sample size formula of Rojas (1964) (Eq. 3-1) uses the sample mean and variance

(incorporated in calculation of k) while the optimal sample allocation procedure (Eq. 3) only uses the sample S. Obviously lowland data, with a considerably larger $S_h$ than upland data (61.3 vs. 8.8) will bias the OSI towards lowland sites. Conversely, upland data, with a lower mean (4.5 vs 22.9), would have a larger value for 1/mean and consequently indicate the need for a larger sample size than for lowland sites. Therefore, determination of a practical sample size must represent a compromise between methods. In consideration of the data presented in this study, 80% and 20% of the samples were allocated to lowland and upland sites, respectively.

Obviously, sample allocation is contingent upon strata accessibility. If dry, use the OSI to allocate samples; if fully flooded, then allocate all samples to upland sites. However, at intermediate flood levels the following conundrum appears: when do I abandon the OSI and allocate more samples to upland sites? For a partially flooded forest, the OSI must be weighted according to the perceived dynamic relative suitability of stratum for habitation (O'Neil and Stimac 1985). This weighting factor ranges from 0 to 1 for unsuitable to suitable habitat, respectively. Because a majority of eggs were found at lowland sites even when Dogwood was ca. 50% inundated (see Fig. 2-2), a majority of samples should be taken at lowland sites until lowland sites are inaccessible. It is possible that the stage-egg

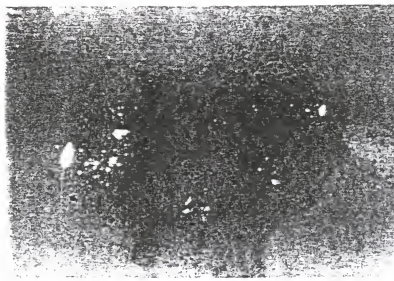
distribution relationship developed in Ch. 2 could be used to allocate samples. Obviously, determination of the appropriate sample allocation is subjectively based on the sampler's knowledge of the site in addition to the methodology proposed here.

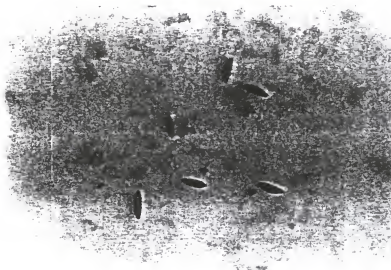
Applications of Sodium Hypochlorite to Mosquito Egg Sampling

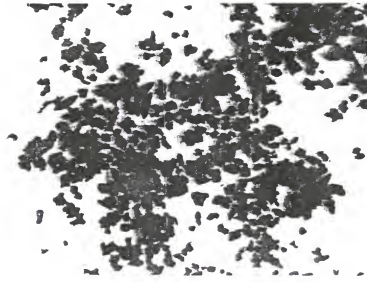
Sodium hypochlorite appears to be an excellent medium for isolating fresh eggs and eggshells from relict eggshells. Significant differences were found between the mean number of eggs/eggshells clearing after a 5 mins exposure to 5% sodium hypochlorite for both fresh eggs and fresh eggshells vs relict eggshells. Only 2 of 100 fresh eggs cleared in 5 min; both of these eggs were infertile. No significant differences were found for the fresh eggs vs fresh eggshells comparison. The time ($\bar{X} \pm SD$) for all eggs to clear completely was $47.8 \pm 6.9$ mins (n = 4 groups of 10 eggs).

This procedure was used to develop a technique for the rapid assay of mosquito eggs in soil. The standard egg assay consists of washing soil through stacked 250 and 150 um sieves, bleaching the soil in the 150 um sieve for 2-5 mins in 50% household bleach, then washing the soil through 150 um sieve to remove bleach and decomposed detritus. Figure 3-3 shows how bleaching improves

Figure 3-3. Applications of sodium hypochlorite to mosquito egg sampling. Unbleached, sieved dirt containing mosquito eggs (top) and after a 5-min exposure to 2.5% sodium hypochlorite (middle). Gryllus sp. feces after a 2-min exposure to 5% sodium hypochlorite (bottom); small, dark particles are egg fragments from eggs ingested by the cricket.







dramatically the ability to distinguish eggs and eggshells from back-ground detritus.

Commercial bleach also appears to be an excellent medium to screen feces for egg fragments. Although egg fragments can be seen in unbleached feces, bleach facilitates the ability to quickly spot suspected egg fragments (Fig. 3-3). Screening must be done quickly because the small egg fragments bleach faster than whole eggs or eggshells.

## Discussion

Horizontal banding characteristic of the distribution of aedine mosquito eggs can be used to design a systematic sampling program which maximizes sampling precision. Calculation of rho using adjacent samples in rows running perpendicular to elevation (and, most importantly, egg density contours) resulted in greater precision than using rows running parallel to elevation contour. But clearly calculations must be made with the distribution in mind in order to maximize sampling precision. Perhaps different systematic sampling patterns would provide greater precision.

Systematic sampling has other advantages over SRS which warrant its use in mosquito egg sampling. Once a sampling grid is established, sample location is easy to

find and sampling can be completed relatively quickly. In SRS the sample location must be determined from a random number table and then the site located. This can be timely and often sites have to be reselected when unsuitable locations are drawn. This is especially true for mangrove sites where trees, logs and roots prohibit sampling. With systematic sampling, an accessible site(s) can be established and sample location quickly established.

The site can be permanent and site-specific population studies can be conducted. Pertinent descriptive data (associated vegetation, topography, water level, soil moisture, etc.) can be collected quickly and may serve as a permanent record for the site. Conversely, a SRS sampling program covering the entire available habitat would require greater resources in order to describe the site. Also, a permanent site sampled over time provides a temporal study of egg population dynamics. Horsfall et al. (1975) systematically sampled a permanent grid over a four-year period to study the population dynamics of Aedes vexans (Meigen) eggs. Various combinations of rows and columns can be used to avoid repeated samples at a specific location; this helps avoid bias due to mechanical damage which is unavoidable when taking soil samples. Even alternative grids can be sampled to minimize damage from sampling.

There are several potential sources of bias associated with systematic sampling. In the strict sense, the

population estimate is only valid for the area sampled. However, the sample site ("grid") is often a subunit of an available sampling universe. Therefore, unless the sampling site is representative of the sampling universe, it is subject to bias. Perhaps the selection of sites without thick tangles of red mangrove prop roots, while more accessible to the sampler, had a significantly different egg population than sites with prop roots. Horsfall et al (1973) found that Ae. vexans oviposited preferentially under dead branches and logs. Unfortunately, sample site selection is ultimately governed by logistics and practicality. Therefore, population estimates must be interpreted with caution. Finally, if the object to be sampled is dispersed in a regular pattern, systematic samples may result in less precision than SRS (Cochran 1963). A sampling interval which coincides with the dispersion pattern would result in high adjacent sample correlation (high values of rho) and less precision. Fortunately, the sampling pattern is flexible, which allows testing for optimal patterns which minimize rho.

Optimal sample allocation proved to be a valuable tool for development of a mosquito egg sampling program. Areas which have known strata featuring different populations may be sampled to produce a single population estimate for the entire site. Sampling can be concentrated in strata with highest variance, thereby maximizing sampling precision and

confidence in population estimates. Obviously, optimal sample allocation need not be calculated for sites with one stratum such as the red mangrove detritus of mixed red and black mangrove forest.

The use of sodium hypochlorite to selectively bleach detritus and egg shells is a tremendous tool for mosquito research. Large samples can be screened for eggs with the naked eye, saving considerable time. Relict eggshells might be aged by bleaching if the relationship between clearing time and eggshell age can be established. Suspected egg predators can be verified by examining the feces for egg fragments (although the method does not apply to piercing-sucking and extraoral feeders).

# CHAPTER 4
# AEDES TAENIORHYNCHUS: A FLUSHWATER MOSQUITO

## Introduction

While aedine mosquitoes are often referred to as floodwater mosquitoes (Horsfall et al.1973) they may, in fact, be more aptly termed flushwater mosquitoes. A floodwater mosquito is one in which hatching of the egg is induced by flooding produced by ponding or a rise in the water table; i.e., the water is brought to the egg. A flushwater mosquito, by contrast, is one in which surface runoff resulting from rain can directly (runoff hatching) or indirectly (eggs flushed to pools) induce hatching; a case of the egg being brought to the water. This curious twist of the traditional floodwater mosquito concept has great potential impact beyond mere scientific novelty. Our approach to modeling, sampling, controlling and understanding aedine mosquitoes will have to consider the flushwater mosquito concept.

Some rather peculiar observations during the summer of 1986 led to the suspicion of egg and larval flushing. Larvae were found in areas suspected to be egg-free and eggs mysteriously disappeared from sites that were not flooded. These observations suggested that eggs and/or larvae were being flushed by surface runoff. A review of the literature revealed that the concept of a flushwater

mosquito, albeit not novel, was poorly understood. Horsfall et al. (1973) documented that runoff hatching does occur in Aedes vexans (Meigen) and suggested that larvae flushed into pools may survive. Filsinger (1941) exposed sods to rain simulated with a bathroom shower and observed flushing of Ae. vexans eggs. However, no quantitative work verifying egg and larval flushing in the field, exploring the relationship of flushing to substrate and elucidating the mechanisms of flushing has been published.

This study addresses the basic dynamics of egg and larval flushing in Ae. taeniorhynchus. Specifically, the objectives of the study were to (1) verify egg and larval flushing in a mangrove forest, (2) test for substrate differences in flushing potential, (3) examine the fate of flushed eggs and (4) examine potential mechanisms of egg and larval flushing such as egg adherence and hatching time.

## Materials and Methods

Verification of egg loss by flushing was obtained in three ways. First, egg populations estimated from surveys conducted before and after heavy rains were compared. Second, egg loss from sites exposed to and protected from rain and runoff were compared. Third, the movement of marked eggs from an oviposition site following a heavy rain was estimated.

Field Studies

Egg and larval surveillance

Surveys were conducted to examine changes in egg and larval populations associated with heavy rainfall. The study site and egg sampling methods are described in Chapter 2. Because it was impossible to predict rain, only one survey documenting changes in an egg population within 24 hours of a storm was obtained. However, several surveys taken at upland and lowland sites following storms provide insight into egg loss due to flushing of different substrates. Population estimates of larvae were obtained from dip counts.

Egg loss from upland soil

Placement of eggs in the field involved a comparison of egg loss in exposed vs covered plots. Plots were only prepared at upland sites because lowland sites were often flooded. Preparation of plots involved removing surface detritus with a whisk broom and then pushing polyvinylchloride (PVC) rings (2.5-cm in diameter and length) into the soil until they were flush with the surface, creating a grid of 12 to 15 rings. Eggs (10 or 20 per ring), collected from wild Ae. taeniorhynchus, were pipetted onto the soil within the ring. Rings not receiving eggs served as controls. The site was then covered with leaf litter. Covered sites were protected from rain by a 30 X 46-cm

sheet of plexiglass suspended over the site on 5-cm diameter PVC legs; a 1.3-cm diameter wooden dowel was inserted through a 1.5-cm hole at each corner of the plexiglass to secure the cover. Additionally, the sites selected were on a mound to minimize the chance that rainfall runoff would move the eggs.

Sites were sampled after 1 to 5 weeks. Rings and soil were carefully removed with forceps and placed in 25 ml plastic vials for transport to the lab. Each vial was then flooded with a yeast infusion and the larvae were counted 24 hrs later. Twelve replications were made and the number of larvae hatching from covered and uncovered sites compared with a 't' test (Schlotzhauer and Littell 1987).

Egg loss from lowland soil

Because lowland substrate consists of red mangrove detritus, egg loss from decaying red mangrove leaves was examined. Decaying red mangrove leaves were placed in an enamel pan with salt water. The pan was then placed in a cage of field-collected, gravid Ae. taeniorhynchus. After 48 hours, leaves were removed and eggs were counted. Leaves were then attached to monofilament fishing line with a fish hook then placed in exposed or covered field sites (Covers were constructed of PVC pipe and plexiglass - see description of those used in upland soil tests.). Surface leaf litter was removed and the leaf was placed gently next

to the moist substrate and then covered with leaf litter. The free end of the monofilament line was tied to a PVC pole. The proportion of eggs lost (arcsine transformed) for covered and uncovered leaves was compared with a 't' test (Schlotzhauer and Littell 1987).

Flushing of marked eggs

A mutant strain of Ae. taeniorhynchus (red eye-spot; obtained from Dr. Richard Baker, Florida Medical Entomology Laboratory, Vero Beach, FL) was used in a field study of the relationship of substrate to movement of eggs by heavy rain. Larvae of this mutant can be easily distinguished from wild mosquitoes by the lack of normal eye pigmentation. Field oviposition involved placement of 200 gravid females in a cage constructed from a 30 X 36-cm white plastic wash tub. The bottom of the tub was removed to expose the underlying substrate. Leaf litter at the junction of the tub and soil was removed and replaced with sandbags made from 10-cm wide surgical stockinette to anchor the cage and minimize openings through which mosquitoes might escape. A natural substrate for oviposition (ca. 15 X 20-cm) was thus created. Gravid mosquitoes (200) were anaesthetized with $CO_2$ and then placed in the tub. A fiberglass screen and plexiglass sheet (36 X 46-cm) were taped on top of the tub with duct tape to prevent exposure to rain and escape of mosquitoes; in a trial run within a 1 $m^3$ cage, only 2 mosquitoes escaped within a 4 day period.

Mosquitoes were allowed to oviposit for 2 days before the remaining mosquitoes were removed with a flashlight aspirator. The cage was then removed and leaf litter replaced. A control that was not exposed to rain was used to estimate the number of eggs deposited.

The distribution and size of mutant mosquito egg populations were estimated following a heavy rain. Sites were sampled within 24 hrs after a rainfall > 2.5-cm (1.0 in). A 10-cm diameter corer and a mason's trowel (10 X 10-cm samples) were used to sample upland and lowland sites, respectively. The following scheme was used: (1) the entire oviposition site was removed intact to provide an estimate of egg loss at the point of origin, (2) a band 10-cm wide above and alongside the oviposition site was sampled to see if eggs moved uphill and laterally, (3) up to 4 rows each (ca. 0.30 m long and 10-cm wide) were sampled downhill from the oviposition site to examine downstream egg movement. Samples were taken to the lab and flooded with yeast infusion. After 24 hrs the number of mutant larvae was counted; mutant larvae were identified by the lack of dark eye-pigment. For large numbers of larvae, the number of mutants was estimated from the proportion found in a sample of 100.

Laboratory Studies of Egg Flushing Dynamics

Laboratory studies were also initiated to investigate the dynamics of egg flushing. Egg-bearing soil was placed in troughs and exposed to simulated rainfall in order to study the dispersal and hatchability of flushed eggs, and the relationship of egg and larval flushing to the duration and intensity of rain. Settling rates for eggs and soil constituents were estimated. Survival of larvae hatched from buried eggs was studied. Flumes were used to study the adherence of eggs to upland soil and red mangrove detritus. Collectively, these studies provide insight into the relationships of substrate and rainfall to egg and larval flushing and the adaptive significance of flushing.

Large trough studies

An oviposition site was created in large wooden troughs (2.0 X 0.5 X 0.5 m) and was exposed to simulated rainfall and runoff. A trough 2 X 0.5 X 0.5 m was constructed from pressure-treated wood; a wooden divider ran lengthwise, creating two adjacent troughs 115 X 25 X 14 cm. Each trough was lined with plastic to prevent water leakage. Adjacent troughs were then carefully lined with either upland or lowland substrate collected with a 25-cm wide flathead shovel. The soil-filled trough was returned to the lab, covered with fiberglass screening to prevent access of mosquitoes and flooded twice to hatch resident

eggs. While flooded, junctions between soil samples were carefully molded by hand to fill in cracks that might trap flushed eggs.

The trough was then prepared for oviposition. A plastic cage secured with sandbags was placed at one end of the trough, creating a 20-cm long oviposition site. One hundred gravid, field-collected Ae. taeniorhynchus were anesthetized with $CO_2$ and placed in the cage to oviposit. The trough was covered with fiberglass screen and incubated for one week at 27°C. Before testing, the screen and cage were removed and the trough was positioned at a 2 to 3° downhill angle on cement blocks. The trough was then exposed to a simulated rain and runoff for 30-min. An oscillating sprinkler (with the oscillation gear removed) was used to produce a steady "rainfall". Rainfall patterns over the ramp and trough were estimated with rain gauges; a composite rainfall rate of 2 to 6 in/hrs (5 - 15 cm/hr) was obtained, a rate typical of summer thunderstorms in Florida (R. Saffle, personal communication). Runoff was enhanced by attaching a 3-m ramp of corrugated fiberglass at the head of the trough to collect and direct additional "rainfall" into the trough. The ramp caused an approximately 15X increase in runoff over the oviposition zone because the ramp is 15X longer than the oviposition zone (3 m vs 0.2-m).

The relative difference in number of eggs and larvae flushed from the oviposition site for upland and lowland soil was addressed in two ways. First, the number of eggs and larvae flushed through a 3.1-cm diameter hole at the end of the trough were collected onto a 150-um sieve. Second, the downstream dispersion of eggs and newly-hatched larvae was compared. After flushing, 15-cm-long sections of the soil were removed, flooded and larvae were counted 24 hr. later. Thus, the functional relationship between egg flushing and distance flushed was elucidated for both substrates.

Mintrough tests

The cumbersome nature of the large trough necessitated the use of smaller troughs (termed minitroughs) in order to increase replication of flushing experiments. Minitroughs were used to study the relationship of egg and larval flushing to storm intensity and duration and to examine the hatching success of flushed eggs. Plastic troughs (20 X 30 X 50 cm) were carefully layered with upland or lowland soil. Approximately 3/4 of the soil surface was covered with plastic which was taped and sandbagged to preclude access by mosquitoes to all surfaces but the exposed soil. A fiberglass screen was taped to the top of the trough and 50 gravid field-collected Ae. taeniorhynchus added. After incubating the mintrough for 1 week, the plastic was

removed and the trough exposed to 1/2-hr simulated rain with runoff enhanced by using a 3-m section of fiberglass. Larvae and eggs flushed out through a 3.8-cm downstream hole were collected in a 10-l bucket at 5-min intervals. Contents were poured through a 150-um sieve; this sieved material was rinsed into a 500-ml plastic deli dish and incubated for 24 hrs at 27° C, then eggs and larvae were counted. The correlation between rainfall (mean rain for 5-min period) and the number of unhatched eggs and larvae (total) flushed from the minitrough was calculated.

The minitroughs also were used to investigate the fate of flushed eggs; i.e., can flushed eggs hatch despite burial? This question was addressed by comparing the hatching success of flushed eggs when subsequently flooded. Minitroughs were exposed to 1/2-hr simulated rainfall and runoff. Half the troughs were incubated for 24 hrs and then flooded carefully with a yeast infusion to induce hatching; the hole was covered with duct tape to prevent leakage. The other minitroughs received no flooding. After 24 hrs the flooded minitrough was carefully drained through a pin hole in the tape to minimize any disturbance that might affect the eggs. The substrate was then cut into quarters, producing two pieces from the oviposition site and two pieces from downstream; eggs from the downstream area presumedly were flushed from the oviposition area. Because the egg sampling procedure (sieving and bleaching -

see Ch. 2) could hatch eggs, the soil pieces were frozen for >24 hrs to kill the eggs before hatched and unhatched eggs were counted. Data from each quarter were used to estimate within treatment variablity.

Several hypotheses were tested with the minitrough data. The proportion of eggs (hatched + unhatched) and larvae flushed through the exit hole, the proportion of flushed eggs that hatched, and the proportion of flushed eggs that hatched when subsequently flooded, were compared for upland and lowland substrate. All proportions were arcsine transformed (to normalize) before analysis with multiple 't' tests; the significance level was dropped from 0.05 to 0.01 to control the experimentwise error rate (Schlotzhauer and Littell 1987).

Hatching and larval survival of buried eggs

At the time of the experiment, it was presumed that buried eggs would not hatch and their hatching percentage would serve as a valid index of the survival of hatchlings. However, Cooney et al. (1981) found that buried Ae. vexans eggs could hatch but larvae were unable to escape. Therefore, a lab study was conducted to examine the relationship of burial depth to hatching and larval survival for buried Ae. taeniorhynchus eggs. Eggs (20) from wild Ae. taeniorhnychus were pipetted into a 10 dram vial and covered with a uniform layer of dry upland soil that had been passed

through a 300-um sieve to remove large particles. A small amount of water was pipetted carefully down the side of the vial to moisten the soil. The vial was gently tapped to create a moist, compact soil. Soil depths of 0.15, 0.25, 0.5, 1.0 and 2.0 cm were used. Water was then pipetted onto the soil until it was moist. After 24-hr, a yeast infusion was pipetted into the vial. The vials were incubated for 24-hr and larvae were counted. The vials were then placed in a freezer to kill the eggs. The soil was sieved and bleached then hatched and unhatched eggs were counted. Controls included (1) placing eggs on top of the soil and then flooding to see if eggs hatched without burial and (2) burying eggs but not flooding to see if eggs hatched proir to flooding. The experiment was replicated 5 times; controls were replicated 3 times. The arcsine transformation of proportions of eggs hatching and larvae surviving was regressed against burial depth.

Settling rates for eggs and upland soil particles

Additionally, the settling rates of eggs and upland soil elements (sand and detrital particles) were calculated to elucidate the likelihood that eggs would be buried. If eggs have the slowest settling rate, then burial would be less likely. Settling rates were measured for 31 individual eggs, sand grains and detrital particles over a 100-cm fall path (50-cm for eggs) in a 250-ml graduated cylinder

(Mehta et al. 1980). Sand and detrital particles were collected by gently running water over the surface of an upland sod sample; the resultant particles represent ele ments which would flush and potentially bury mosquito eggs. The settling rates for eggs, sand and detritus were compared with a multiple 't' test (Schlotzhauer and Littell 1987).

Adherent properties of eggs

Adherence to red mangrove detritus. The adherence of Ae. taeniorhynchus eggs to red mangrove detritus was examined. Partially-decayed red mangrove leaves, collected from immediately below the surface litter, were laid flat in an enamel pan to which a small amount of pupal water (water from which adults had emerged) was added as an oviposition attractant (Ikeshoji and Mulla 1970). The pan was placed in a cage containing gravid, field-collected Ae. taeniorhynchus. After 48-hr, the pan was removed and egg-bearing leaf pieces were excised with a razor blade. The bottom of the leaf piece was blotted dry on tissue paper then glued to the plexiglass with Superglue® by appressing the leaf with forceps. Ten to 12 pieces were glued to a sheet. The sheet was then placed in a styrofoam cooler in which grooves were cut to hold the sheet in place; a moist paper towel was added to maintain high humidity. Leaves

dried but only infertile eggs collapsed. It was thought that this would realistically represent field conditions during hot, dry weather. Immediately before testing, the number of eggs per leaf were counted with a dissecting microscope.

Adherence was tested by exposing egg-bearing leaves to a water current in a flume. The plexiglass sheet with eggs was secured to the inner wall of a flume (Lott 1986) with two C-clamps and exposed to a water current for 15 min. Water velocity was estimated from the mean of readings taken adjacent to the plexiglass at 2, 7 and 14 min with a Marsh-McBirney model 523 electromagnetic water current meter. One plexiglass sheet was not placed in the flume and served as a control for eggs lost in handling.

Because leaf pieces dried before gluing and egg counting could be completed, a smaller flume that allowed leaves to be tested rapidly was developed to assess egg adherence to wet leaves. The flume consisted of a 3 X 1 X 0.025 m piece of Tuff-R® fiberglass insulation with two strips of silicone caulk 2.5-cm apart running lengthwise to create a channel for water runoff. The ramp was positioned at a slight incline to control the speed and direction of the water current. One end of a section of rubber tubing was attached to a faucet and the remaining end was attached to the elevated end of the channel with duct tape. Current in the channel was estimated by timing confetti (1

mm diameter circles of notebook paper made with a paper punch) floating the last 0.5 m of the channel; 10 reps were made over the course of an experiment. Mean current velocity was calculated by multiplying mean confetti velocity by 0.85 to account for loss in current velocity due to drag (A. J. Mehta, personal communication). Egg adherence was tested by pinning an egg-bearing leaf piece to the channel bottom with a #3 insect pin at the downstream end of the channel and exposing the leaf piece to a current for 60 secs. A 150-um sieve was used to collect flushed eggs. Unflushed eggs were counted under a dissecting microscope. The proportions of eggs flushed (arcsine transformed) from wet and dry leaves for different current velocities were compared using a one-way analysis of variance procedure.

Adherence to upland soil. Egg adherence to upland soil was tested in the large flume. Sections of upland soil were placed in a 46 X 15 X 5 cm plastic tray and then exposed to gravid field-collected Ae. taeniorhynchus for oviposition. Ten mosquitoes were confined under a 5-cm dia. plastic cup inserted into the soil at ca. 10-cm intervals. The tray was incubated at 27° C and high relative humidity for two weeks.

Soil samples were then prepared for testing. The cup was removed from each section and a 10 X 10 X 5 cm piece of soil was removed carefully and placed within a similarly-sized

sheet metal box (sample box). The sample box was then placed midway in a plastic tray (46 X 15 X 5 cm) in which the exposed ends were sealed with duct tape. The tape adjacent to the sample was folded over, creating a flap that reduced turbulent flow across the sample yet allowed the sample box to be removed easily. The tray and sample box were then placed on the flume bottom ca. 1 m from the outflow.

The following device was prepared to house the sample-containing tray and to minimize turbulent flow. Two 46 X 15 X 5 cm plastic trays were placed upside down immediately ahead of the sample tray and secured to the flume bottom with duct tape. Ahead of these trays, a triangular ramp (basal length = 46 cm, height = 15 cm), constructed of Tuff-R® insulation, was connected to the flume bottom to provide a smooth ascent for inflowing water. A similar but shorter ramp (basal length = 31 cm) was placed downstream from the sample tray to reduce outflow eddy turbulence. The trays and ramps were then coupled and attached to the flume bottom and sides with duct tape, creating a smooth, uniform surface that minimized turbulent flow over the exposed soil sample.

Samples were placed in the flume and exposed to current for 15 min. Depth above the sample was 1.5-2.0 cm. Current speed was measured immediately above the sample at

2, 7 and 12 min with the current meter; the mean of the three measurements was used in calculations. Currents ranging from 3 - 37 cm/sec. (0.1 - 1.2 ft/sec) were used. Hatched and unhatched eggs were counted after freezing, sieving and bleaching. Five samples that were not placed in the flume served as controls. The number of eggs/sample was divided by the estimated number of control eggs (mean of 5 reps) to determine the proportion of eggs adhering. This was regressed against current velocity squared (current velocity$^2$ is proportional to the power of the current (A. J. Mehta, personal communication)) to show the relationship between egg flushing and current speed.

## Results

### Field Verification of Egg Flushing

#### Egg and larval surveillance

While data from egg surveys are primarily descriptive, egg flushing and differences between lowland and upland substrates were noticeable. Two egg surveys conducted on 14 and 16 July, 1986 at two upland Dogwood sites produced (mean $\pm$ SE) 13.94 $\pm$ 2.57 (n = 55) and 12.36 $\pm$ 2.24 eggs/sample (n = 53), respectively. On the evening of 16 July, 1986, 1.6 cm of rain fell during a brief but heavy thunderstorm. An upland survey the next day produced only 0.59 $\pm$ 0.31 eggs/sample (n = 53). Similarly, two upland surveys (21 Aug. 1986) conducted after 4.6 cm of rain fell

during the previous 72 hrs. indicated low egg populations ($0.52 \pm 0.25$ and $0.56 \pm 0.30$ eggs/sample; n = 56 and 54, respectively). These data suggest that substantial decreases in upland egg populations may occur following heavy rain.

Surveys conducted in August, 1986, and March, 1987, indicated that flushing may have less impact on lowland egg populations. A dry period in late July to early August was broken by 5.2-cm rain on 11 Aug. Egg surveys conducted on 12 Aug. produced $12.40 \pm 4.01$ eggs/sample (n = 40) at a lowland site but only $1.78 \pm 0.71$ eggs/sample (n = 32) at a nearby upland site. Similar egg surveys were taken on 3 Mar., 1987 after 5.2 cm of rain fell during the previous 48 hr. The lowland (n = 48) and upland (n = 48) surveys produced respective means of $30.67 \pm 5.25$ and $0.56 \pm 0.28$ eggs/sample. Oviposition preferences probably do not account for the nearly 55 fold difference in egg population. Water table records at Dogwood indicate that much of the lowland substrate was flooded during the peak oviposition period for this particular mosquito brood and that substantial upland oviposition was possible. These data strongly suggest that egg flushing is greater at upland than at lowland sites.

Nonetheless, egg flushing can occur from lowland substrates. Data from egg and larval surveys conducted sequentially at a lowland site before and after a heavy rain suggested that larvae may also be flushed. An egg survey

at a lowland Dogwood site on 3 June 1986 (n = 74) produced a 62.9 ± 28.2 eggs/sample in thick detritus substrate but no eggs from lower elevations featuring little leaf litter (a surface contour map of the egg population is shown in Ch. 2, Fig. 2-6). Only a few hours after this egg survey, the lowest (and presumedly egg-free) elevations were flooded following 1.65 cm of rain. Interestingly, 1st instar Ae. taeniorhynchus larvae were observed; 30 dips produced 24.60 ± 4.59 larvae/dip. These data suggest that either eggs flushed into lower sites and hatched or that eggs hatched when overwashed by surface runoff, flushing larvae into downstream pools. It is likely that both phenomena occurred. Horsfall et al. (1973) observed flushing of teneral Ae. vexans larvae in surface runoff following heavy rain. Filsinger (1941) observed that Ae. vexans eggs were flushed from sods experimentally exposed to simulated rainfall.

Larval surveys suggest that flushing from upland sites at Dogwood may produce larvae despite little increase in the water table. During June and July, 1986, several heavy rains filled the Dogwood basin. As a result, additional rainfall simply overflowed into adjacent basins, the water table remained stable and little additional flooding occurred. Nonetheless, larvae were found along the shore. Figure 4-1 summarizes water table, rainfall and larval counts for this event; note that the last larval brood is

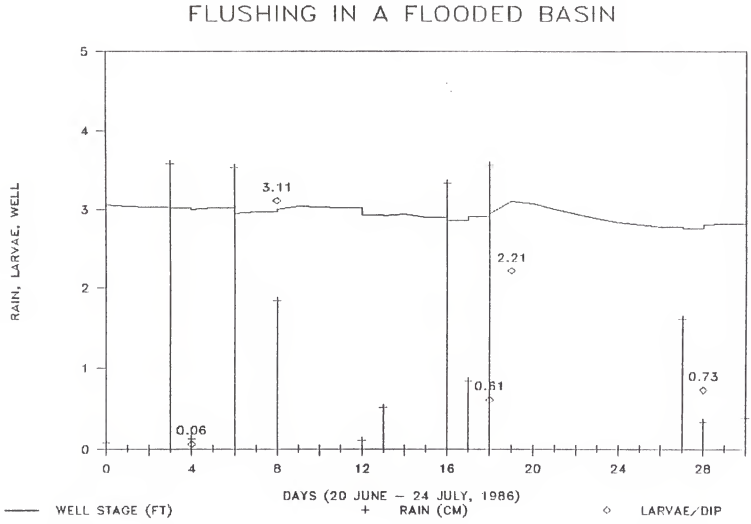



Figure 4-1. A plot of Aedes taeniorhynchus larval populations presumedly hatched by runoff. Note that the Dogwood water table increased very little despite heavy rain.

concurrent with the decreased upland egg population documented for 14 to 17 July 1986. While the mean number of larvae/dip is small, the actual population may be larger. Dips taken away from the shoreline usually contained no larvae, thus reducing the mean; some dips taken along the shoreline had >50 larvae. Considering the perimeter length of the shoreline, substantial larval populations may hatch due to flushing.

Egg loss from upland soil

Uncovered sites lost significantly more eggs than covered sites. Table 4-1 shows the results of the 12 trials run during 1986-87. Overall, the covered and uncovered sites respectively lost 44.2 $\pm$ 26.7% and 77.1 $\pm$ 22.0% of the eggs emplaced. The mean number of larvae hatching per test was significantly different ($P < 0.01$) in 8/12 (67%) trials. These results suggest that significant egg flushing occurs at upland sites.

The ring test results also suggest that rainfall and season may be important factors controlling egg flushing. Heavy rainfall during summer experiments (May to August) resulted in nearly 100 % loss of uncovered eggs. However, comparably heavy rains in winter (February to March) produced losses of 50.0 and 64.9 %. Perhaps winter temperatures reduce egg loss due to runoff hatching by retarding the hatching rate of potentially diapausing eggs; Parker (1985) and Ritchie (see Ch. 6) found that the percent hatch

Ae. taeniorhynchus eggs decreased in response to lower temperatures during the winter.

Table 4-1. Aedes taeniorhynchus egg loss from PVC rings placed in upland soil at Dogwood from July 1986 to July 1987. Ditto marks refer to replicate experiments. First 5 tests used 20 eggs/ring; next 7 tests used 10 eggs/ring. Significant treatment differences tested with 't' test.

| Date in, date out | No. eggs | Percent lost Covered | Uncovered | Signi. at 0.01 level |
|---|---|---|---|---|
| 22 July, 6 Aug. | 200 | 13.9 | 62.2 | yes |
| 7 Aug., 21 Aug. | 200 | 52.8 | 100.0 | yes |
| " " | 200 | 76.8 | 99.7 | yes |
| 5 Oct., 12 Oct. | 200 | 42.2 | 80.3 | yes |
| " " | 200 | 38.6 | 77.3 | no |
| 3 Jan., 11 Jan. | 150 | 13.5 | 38.9 | yes |
| 21 Feb., 5 Mar. | 90 | 36.2 | 57.4 | yes |
| " " | 60 | 9.6 | 50.0 | no |
| 21 Feb., 29 Mar. | 60 | 58.5 | 64.9 | no |
| 25 April, 19 May | 150 | 54.9 | 94.2 | yes |
| 10 June, 1 July | 120 | 33.3 | 100.0 | yes |
| 17 June, 1 July | 120 | 100.0 | 100.0 | no |

Egg loss from lowland soil

Results from the covered and uncovered egg-bearing leaf study suggest that rainfall may reduce egg populations on mangrove detritus. For the five covered leaves recovered, 206 of the 476 (43.3%) original eggs were still attached. For the seven uncovered leaves, 86 of 612 (14.1%) of the original eggs were recovered, a significant loss ($P = 0.016$). Interestingly 39 (45.3%) of the recovered eggs had hatched. This suggests that eggs on leaves may be flushed and can hatch, probably when exposed to runoff. Additionally, hatching has been observed in eggs glued to leaves with silicone caulk despite no inundation; runoff hatching might account for this observation.

Flushing of marked eggs

Unfortunately, only five field tests were completed in which mutant mosquitoes were used. On two occasions, study sites were completely inundated before sampling, and on another, the site was inadvertantly treated with insecticide. The results of the tests are shown in Table 4-2. The number of marker larvae counted 24 hrs after flooding the control was 3759.

Table 4-2. Movement of Aedes taeniorhynchus (red eye-spot strain) eggs from oviposition site after heavy rain.

I. Lowland sites

| Date: | 12 June | 23 August |
|---|---|---|
| Rainfall (cm): | 5.03 | 2.79 |
| # marked larvae (%) hatched from site | | |
| Oviposition: | 995 (93.7) | 1681 (99.7) |
| 10-cm downhill: | 47 ( 4.5) | 2 ( 0.1) |
| 20 " " | 18 ( 1.7) | 3 ( 0.2) |
| 30 " " | 1 ( 0.1) | 0 |
| 40 " " | flooded | flooded |

II. Upland sites

| Date: | 12 June | 13 Aug. | 13 Aug. |
|---|---|---|---|
| Rainfall (cm): | 5.03 | 2.84 | 2.84 |
| # marked larvae (%) hatched from site | | | |
| Oviposition: | 268 (77.8) | 75 (100) | 16 (100) |
| 10-cm downhill: | 0 | 0 | 0 |
| 20 " " | 0 | 0 | 0 |
| 30 " " | 81 (23.2) | 0 | 0 |
| 40 " " | 0 | 0 | 0 |

While the number of replications was insufficient for statistical analysis, some trends are noticeable. First, more eggs were lost at upland than at lowland sites; second, a graded distribution of eggs downstream from the oviposition site was not apparent.

## Laboratory Studies of Egg Flushing Dynamics

### Large trough studies

Enhanced runoff from the 3-m runoff ramp increased larval flushing for upland soil (Table 4-3). Additionally, upland sites had greater downstream dispersion and larger number of larvae flushed out of the trough than did lowland substrates. Upland soil is more compacted than lowland and is therefore more susceptible to runoff; puddling has only been observed on upland soil.

Table 4-3. Larvae flushed from lowland and upland red mangrove soil in large troughs exposed to 30 min simulated rain. Larvae expressed as percentage of (larvae flushed + larvae hatched from soil sections).

| Site downstream in cm | no runoff ramp (3 replications) lowland | upland | 3-m runoff ramp (2 replications) lowland | upland |
|---|---|---|---|---|
| Ovip | 79.2 | 67.9 | 86.4 | 44.3 |
| 15 | 14.3 | 12.4 | 7.4 | 11.8 |
| 30 | 2.5 | 7.8 | 1.9 | 7.6 |
| 45 | 1.0 | 4.0 | 0.9 | 2.5 |
| 60 | 0.3 | 0.3 | 0.9 | 1.9 |
| 75 | 1.5 | 1.4 | 0.6 | 2.6 |
| out flush | 2.8 | 6.3 | 2.0 | 29.4 |

### Minitrough tests

Flushing experiments with the minitroughs also demonstrated that egg and larval flushing is greater on upland than on lowland soil. The mean percentage (100 X number of eggs or larvae/total number of eggs found in the minitrough) of eggs flushed out of the minitrough was 16.7 (SD

= 21.5, median = 12.3, n = 10) and 2.4% (SD = 2.7, median = 0.7, n = 7) for upland and lowland substrates, respectively; a significantly different mean ($P < 0.01$). Hatched eggs accounted for 56.1 % of eggs flushed out. The mean percentage of larvae flushed out was 30.4 (SD = 17.6, median = 31.8, n = 10) and 3.3% (SD = 6.1, median = 0.6, n = 7) for upland and lowland substrates, respectively; also significantly different ($P < 0.01$).

Larvae were quickly flushed after exposure to simulated rain and runoff. Figure 4-2 shows the cumulative proportion of total larvae flushed from upland soil during consecutive 5 minute periods of exposure to a 30 min. simulated rainfall. Clearly, runoff hatching and larval flushing are initiated soon after rainfall. The data suggests that even brief heavy rains may result in hatching and could possibly strand many larvae. It would have been interesting to continue simulated rainfall to determine the time when flushing is complete. Curiously, no significant correlation ($P > 0.50$) was found between mean rain intensity and the proportion of larvae flushed from the trough. Perhaps a rain intensity threshold had been reached. More likely, however, is that hatching was a somewhat random event based upon chance exposure to runoff, because a uniform sheetflow runoff was not observed in upland mini-troughs.

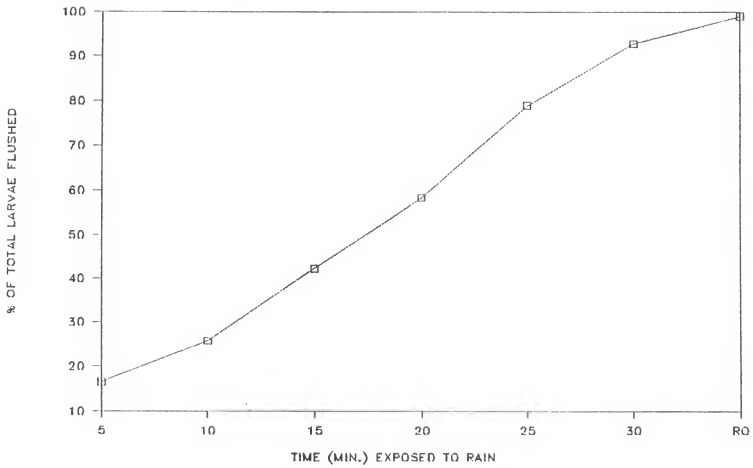



Figure 4-2. A plot of the cumulative proportion of larvae flushed out of a minitrough exposed to a 30-min simulated rain. A 3-m fiberglass ramp was attached to the elevated end of the minitrough to increase the volume of runoff; RO refers to the final runoff after the rain simulation was ended.

Minitrough experiments also indicated that unhatched eggs that are flushed and possibly buried can hatch when flooded (Table 4-4). The proportion of flushed eggs that hatched before and after flooding was significatly different (P = 0.0001 and 0.007, respectively) for upland and lowland soil. The lower hatching percentages for lowland soil, while not significantly different than upland (P = 0.041 and 0.075 for flush and for flush and flood treatments, respectively) suggest that thick detritus may inhibit eclosion. Unfortunately the limited number of replications precludes drawing strong conclusions. However, the large number of eggs/minitrough (over 1000) does give credence to the results. Additionally, the fate of larvae

Table 4-4. Fate of Aedes taeniorhynchus eggs after flushing. Data is the percentage (mean ± SD) of eggs hatched to eggs found in the oviposition zone and the downstream zone for upland and lowland soils exposed to either flooding or no flooding after exposure to 30 minute simulated rainfall. Five replicates/test, except only two replicates for lowland flush + flood. The values should be interpreted as follows: numbers under the heading "Flush only" represent the percentage of eggs that have hatched after only being flushed; the numbers under "Flush and flood" are the percentage of eggs that have hatched after exposure to flushing and, 24 hrs. later, flooding.

| | % hatch by soil type and location in trough | | | |
|---|---|---|---|---|
| | upland zone | | lowland zone | |
| Treatment | ovip. | flush | ovip. | flush |
| Flush only | 29.4±12.7 | 51.1±18.0 | 11.1± 7.6 | 37.1± 9.7 |
| Flush + flood | 94.5± 5.4 | 93.0± 4.1 | 79.1±11.5 | 79.5±18.5 |

from these eggs is unknown because larvae were not counted. However, results from the laboratory studies on egg settling rates and the hatching of buried eggs provide insight into the likelihood of egg burial and the fate of larvae hatched from flushed eggs.

Hatching and larval survival of buried eggs

Hatching was readily induced in buried Ae. taeniorhynchus eggs but larvae had difficulty escaping as soil depth increased. While hatching was >50% for all buried eggs, larvae did not escape if eggs were buried >0.50-cm (Fig.5-3); regression of burial depth vs proportion of eggs hatching was not significant ($P > 0.50$) while burial depth vs larval survival was ($P < 0.001$). Cooney et al. (1981) made similar observations of Ae. vexans eggs in plowed fields. Microscopic examination of buried eggs showed that interstices between sand and detritus grains permitted cracking of the chorion and, in some cases, separation of the operculum. However, the larva could escape from neither the egg shell nor the soil. Controls indicated that some buried eggs hatched without flooding; perhaps hatching had begun during handling, or soil moisture was sufficient to induce hatching.

The results of this study invalidate the use of egg hatching as an index of egg (ultimately larval) survival. Clearly buried eggs hatch normally but larvae may not

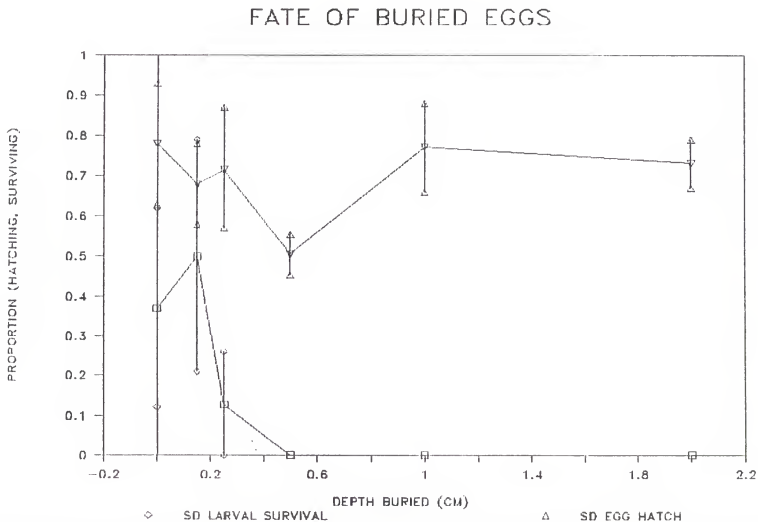



Figure 4-3. A plot of the mean (± SD, n = 8) percent hatch (top line) and larval survival (bottom line) from eggs buried in upland soil and then flooded with a yeast infusion.

escape. Therefore future studies of egg flushing should concentrate on larval survival. Nonetheless, the slow settling rate and rapid hatch of Ae. taeniorhynchus eggs suggest that deep burial of unhatched eggs is unlikely.

Settling rates for eggs and upland soil particles

Sand and organic detritus particles settled at significantly faster rates than did eggs (Table 4-5), although one detritus particle had a settling rate (2.79-cm/sec.) comparable to that of the eggs. Generally, the data suggest that suspended sand and detritus would settle before eggs would, reducing the probability of egg burial.

Table 4-5. Results from settling rate tests for sand, organic detritus and Aedes taeniorhynchus eggs. Settling rate (velocity) for a 100-cm fall (sand, detritus) or a 50-cm fall (eggs) were conducted in tap water in a 250 ml. graduated cylinder at room temperature. Data (n = 31) were analyzed with a 't' test.

| | Sand | Detritus | Eggs |
|---|---|---|---|
| Mean velocity in cm/sec.: | 20.85 | 16.49 | 2.97 |
| SD | 4.93 | 16.10 | 0.87 |
| SE | 0.89 | 2.89 | 0.16 |
| Statistics | Sand vs. detritus | Sand vs. egg | Egg vs. detritus |
| t value | 1.22 | 13.62 | 11.43 |
| P | 0.20 - 0.50 | < 0.001 | < 0.001 |

Conversely, suspended sand and detritus could bury eggs as they fall out of suspension due to a decrease in runoff velocity. Eggs suspended in runoff leading into pools of standing water could settle and be buried by later influxes of soil particles. This might explain why eggs hatch rapidly when exposed to runoff in order to avoid burial by settling sand and detritus particles.

Adherent Properties of Eggs

Adherence to red mangrove detritus. Egg adherence to red mangrove detritus (dead leaves) was highly dependent upon leaf moisture. Results of flume tests using dry and moist leaves indicate that drying of the leaf significantly increases egg adherence. A one-way analysis of variance procedure was used to test for significant differences among the proportion of eggs lost (arcsine transformed) for the following treatments: dry leaf exposed to 0 (control), 9, 18, 27, 43, 67 and 79 cm/sec current and wet detritus exposed to 9 cm/sec current. Faster currents were not used with wet detritus because 97.4% of the eggs were flushed by the slowest current. The results are shown in Table 4-6. No significant differences were found for proportion of eggs flushed for the seven dry leaf treatments. However, addition of wet leaf data did result in significant treatment differences ($P < 0.001$). The results suggest that eggs are tightly bound as leaf detritus dries. While

prolonged runoff could increase leaf wetness and potentially reduce egg adherence, no loss of adherence was noted over the 15-min test exposure despite some leaf wetting.

Table 4-6. Adherence of Aedes taeniorhynchus to red mangrove detritus: percentage of eggs lost (flushed) when exposed to different water current speeds. Exposure to 0 current represents control to measure egg loss from handling.

| Current (cm/sec) | Dry leaf reps | Dry leaf mean | Dry leaf SD | Wet leaf reps | Wet leaf mean | Wet leaf SD |
|---|---|---|---|---|---|---|
| 0 | 15 | 7.5 | 0.1 | - | - | - |
| 7 | - | - | - | 15 | 97.4 | 3.8 |
| 9 | 9 | 1.6 | 2.5 | - | - | - |
| 18 | 8 | 2.3 | 4.2 | - | - | - |
| 27 | 14 | 13.0 | 9.4 | - | - | - |
| 43 | 14 | 13.7 | 9.1 | - | - | - |
| 67 | 12 | 4.2 | 3.9 | - | - | - |
| 79 | 14 | 10.0 | 9.1 | - | - | - |

Adherence to upland soil. Adherence of Ae. taeniorhynchus eggs to upland soil was inversely proportional to current velocity$^2$. Figure 4-4 shows the regression of proportion of eggs adhering vs current velocity squared; the regression equation proportion eggs adhering = 93.11 - 0.065 X (current velocity squared in (cm/sec)$^2$) was significant ($P < 0.001$). However, variablity was high resulting in an $r^2$ of only 0.48. The variablity can be traced to the use of

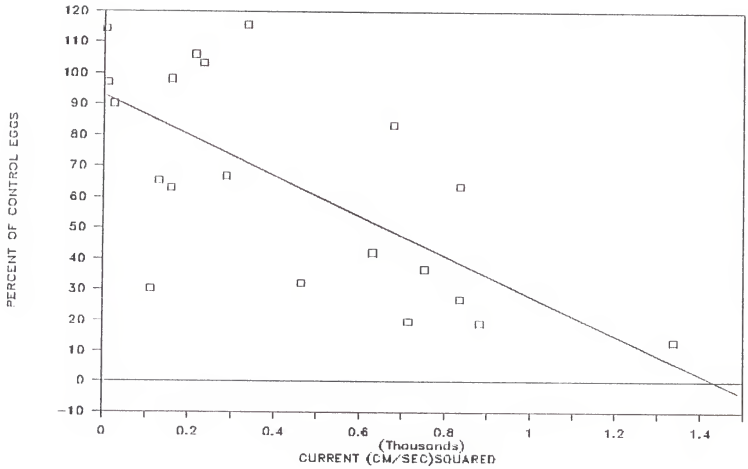



Figure 4-4. A plot of the proportion of Aedes taeniorhynchus eggs adhering to upland soil vs current velocity squared for tests conducted in a closed-ended flume (Lott 1986). Egg proportion expressed as number of test eggs/mean number of control eggs; current is mean of three measurements taken at 5 min intervals during a 15-min period. The regression line, Y = 93.11 - 0.065X, is also shown.

estimates as variables; neither the true number of eggs adhering nor the true water current are known, a clear violation of the assumption that the independent variable is measured "without error" (Marks 1982). Thus, while the data cannot provide a statistically valid estimate of egg adherence, it does illustrate the relationship of egg adherence and water current.

The data suggest that a current in excess of 46 cm/sec may flush all eggs. Values approaching 91 cm/sec (3 ft/sec) might be expected during storm runoff in mangroves (A. J. Mehta, personal communication). Also, runoff hatching was observed during the flume tests; 20. $\pm$ 17.6% of the adhering eggs hatched. Clearly, prolonged runoff may reduce egg populations significantly in some soils due to runoff hatching and egg flushing.

## Discussion

The results establish two important factors regarding egg and larval flushing. First, flushing involves both runoff hatching and egg dispersal. Second, substrate porosity (i.e., the ability of a soil to produce runoff) appears to be related to the magnitude of egg and larvae flushing. The compacted, sandy soil from upland areas of Dogwood produced significantly greater egg and larval flushing than the porous, detritus-rich lowland substrate. Field observations during thunderstorms indicate that upland areas were covered by sheetflow runoff and puddles whereas runoff at

lowland areas percolated through leaf litter. Thus, eggs on compacted soils are more likely to be directly exposed to water; it is here that Ae. taeniorhynchus becomes a flushwater mosquito.

The implications of the flushwater mosquito concept are many. Construction of simulation models, egg and larval sampling, mosquito-control strategies and current concepts regarding mosquito ecology may be affected.

The flushwater mosquito concept will have application to the construction of simulation models for aedine mosquitoes. Floodwater mosquito oviposition, egg distribution and hatching are often assigned to vertical strata corresponding to elevation (Focks et al. 1988a; see Ch. 6). As the water table rises and floods higher strata, eggs within the flooded strata are hatched. This study suggests that a significant proportion of eggs in higher yet unflooded strata would hatch, the exact proportion being a function of rainfall, season, substrate, etc. Survival of larvae hatched by runoff will depend on the probability of reaching standing water, in addition to factors such as longevity of standing water and predation. This approach will be used to construct the simulation model of Ae. taeniorhynchus presented in Ch. 6.

The flushwater mosquito concept is applicable to modeling using buildup-washoff models. Heaney (1986) modeled the flushing of sediments from street surfaces

caused by runoff due to heavy rain. The procedures developed to describe the dynamics of sediments subjected to buildup-washoff could be applied to flushwater mosquiuto eggs.

The flushwater mosquito concept will become increasingly important as basins fill with water. Additional rainfall results in only minor increases to pond depth as water spills over into adjacent basins. Under these circumstances, which can be quite common during Florida's rainy season, eggs laid above the basin water level may be the only eggs that hatch. Figure 4-1 shows several such incidents during the summer of 1986. Clearly, the data indicate that substantial changes in egg and larval populations result from flushing despite little change in water level.

The flushwater mosquito concept has application to larval sampling and surveillance. First, inspectors will have to consider that larvae may be flushed to areas that previously contained no eggs. Second, flushed larvae will tend to be concentrated along the shoreline where runoff intercepts standing water, especially in fully-flooded basins. Thus, knowledge of egg and larval flushing can help define larval distribution.

The flushwater mosquito concept has application to control. Obviously, any improvement in surveillance aids mosquito control programs. Perhaps more importantly, new

application strategies leading to more efficient control might be developed. Pond margins could be targeted for larviciding. Surface runoff could be used for passive delivery of pesticide to larvae. In such a case, granular pesticides might be preapplied to unflooded areas to await activation and dispersion by flushing. Certainly other applications exist.

Finally, the flushwater mosquito concept has application to mosquito ecology. Understanding of the adaptive advantages of flushing improves our understanding of the forces that drive aedine mosquito population dynamics. Certainly flushing represents the only way for eggs laid above the highest floodline to produce surviving larvae. This is surely a selective advantage to aedine mosquitoes in wet climates where basins are often full so that rainfall results in spillover rather than new flooding.

Additionally, Ae. taeniorhynchus eggs and larvae are subject to high summer mortality. Survival rates of less than 50% per week were found for eggs at Dogwood during the summer (see Ch. 5). Larval survival should decrease after incipient flooding as both predator size and populations increase (Focks et al. 1988a; see Ch. 6). Thus, the risk of predation for eggs and larvae increases as the time awaiting hatch increases. If this risk, on average, is greater than the additional risk of a runoff-hatched larvae being stranded, then runoff is adaptively advanta-

geous. Also, flushed eggs that do not hatch have an adaptive advantage; such eggs are closer to standing water and more likely to be flooded than nonflushed eggs.

# CHAPTER 5
# NATURAL MORTALITY AND PREDATORS OF AEDES TAENIORHYNCHUS EGGS IN A MANGROVE FOREST

## Introduction

Valid estimates of the natural mortality of Aedes taeniorhynchus eggs were necessary for construction of the simulation model, TAENISIM. Senescence, predation and flushing (see Ch. 4) are sources of egg loss in the field. Natural senescence of aedine mosquito eggs appears to occur at very low rates; Psorophora columbiae (Dyar and Knab) egg senescence was found to range from 1 to 2% per week (Olson and Meek 1979). This suggests that predation may have more impact on mortality than does senescence. This study quantifies the impact of predation on natural populations of Ae. taeniorhynchus in a mangrove forest.

Unfortunately, only limited published information concerning field mortality and predators of aedine mosquito eggs is available. Mortality of Ae. taeniorhynchus eggs is apparently highest in the summer (Bidlingmayer and Schoof 1956, J. H. Frank, unpublished data). Ants (James 1966; D. Lee, personal communication), carabid beetles (James 1966, Buxton and Yates 1939) and cicindelid beetles (J. H. Frank, personal communication) have been observed to eat aedine mosquito eggs. However, the lack of standard methodology necessitated the development of methods to estimate natural

egg mortality and to identify potential egg predators. The objectives of this study were threefold; (1) to develop a method to quantify egg mortality in the field for upland (compacted, fine-grained soil) and lowland (detrital peat) substrates at Dogwood, (2) to estimate egg mortality rates at these sites over the course of a year, and (3) to investigate the role of the mangrove fauna as Ae. taeniorhynchus egg predators.

## Materials and Methods

### Field Estimates of Egg Mortality

Mortality of Ae. taeniorhynchus eggs was estimated for upland and lowland substrates at Dogwood. At upland sites, egg mortality was estimated by the disappearance of eggs placed atop soil within PVC rings that were inserted into the soil (referred to as the ring test). Five randomly selected rings received no eggs and served as controls for resident eggs and oviposition. For lowland sites, egg mortality was estimated by determining the number of eggs lost from dead mangrove leaves on which eggs were glued with silicone caulk (referred to as the leaf test). Specific details concerning egg placement for both methods are presented in Ch. 4.

Samples were processed as follows. Rings were collected by carefully removing surface litter with forceps, and then individually placing rings and accompanying soil

(including remaining test eggs) in 25-ml plastic vials for transport to the lab. The vial was incubated at 27° C for 24 hr, and then flooded with yeast infusion to induce hatching. Larvae were counted for each vial ca. 24 hr later; soil was saved for later analysis.

Two methods were then used to estimate egg mortality. In method A, egg mortality was estimated by subtracting the mean number of larvae per ring from the expected mean number of larvae per ring (i.e. no egg predation). The number of larvae per ring was corrected for resident eggs by subtracting the mean number of larvae hatching from the control rings. The mean expected number of larvae/ring was calculated by multiplying the number of eggs originally placed in the ring by fertility, hatching and larval survival proportions to correct for sources of "egg loss". Fertility was determined by clearing a sample ($n > 100$) of eggs used in the test. Hatching was assumed to be a constant 0.95 based on field estimates (Ch. 6). Larval survival, estimated from a control conducted in a predator-free environment, was assumed a constant 0.95.

The second method (B) involved fewer correction factors. Rings were processed as above. However, the ring soil was sieved and bleached and the number of viable eggs was counted (viable eggs consisted of hatched eggs and unhatched eggs with normal embryos). The new value was then divided by 0.90, the sampling efficiency of the sieve

and bleaching method, to estimate the number of viable eggs. This value was divided by the total number of eggs originally placed in the field to provide an estimate of egg survival. Weekly survival and mortality rates were calculated. Appendix C shows a sample calculation using both methods.

While both methods produce similar egg mortality estimates, method B is preferred. Eggs can be quickly processed and easily tallied by pooling the ring soil. The larval count for each ring can be used to estimate sample variance. The method only involves one "correction" factor (egg sampling efficiency) whereas method A involves three (egg fertility, hatching and larval survival), increasing the probability of error.

Egg mortality was much easier to estimate for lowland experiments. Because eggs were glued to leaves, a direct count of the number of viable eggs remaining after field exposure was made using a microscope. Eggs that had hatched during exposure were excluded from the data. In one experiment, between-site variation in egg mortality was tested by clumping five egg-bearing leaves at five different lowland sites. The variation in the proportion of eggs lost per leaf (arcsine transformed) within and between sites was compared with a one-way analysis of variance test (Schlotzhauer and Littell 1987).

Identification of Potential Predators of Eggs

Several experiments were used to examine the potential of selected mangrove fauna to consume Ae. taeniorhynchus eggs. The experimental method was intended to furnish the animal relatively uncrowded conditions and a choice of food while providing the researcher with an easily replicated procedure to determine egg predation accurately. Small animals were tested in 25-ml plastic vials and larger animals, such as crabs, were tested in 10-liter buckets. When possible, social species, such as ants, were colonized before testing. Mosquito eggs and red mangrove litter (dead leaves, twigs) were provided to test animals to differentiate detritivores from carnivores/omnivores that may eat mosquito eggs. A comprehensive list of all animals tested is provided in Appendix D.

Animals were collected in a variety of ways. Animals were removed from red mangrove detritus by hand or with a Berlese funnel. Animals were also collected by flooding mangrove soil collected for mosquito egg sampling (Ch.2) and flushing experiments (Ch. 4). This proved to be the most efficient method; soon after flooding, large numbers of insects and other arthropods surfaced and could be readily collected by hand or with a dip net or paint brush. Ant colonies were collected in situ, then placed in buckets coated with Fluon® to prevent escape. Crabs and snails were caught by hand.

Small animals were tested in 25-ml plastic vials; each 1/3 filled with plaster of Paris that was moistened with distilled water to maintain high relative humidity. A small section of moist red mangrove detritus and a red mangrove twig were placed in a 25-ml plastic vial containing one test animal, and the vial was capped. After 24 hr, either 10 or 20 Ae. taeniorhynchus eggs were added. After 48 hr, the animal, detritus, and vial were rinsed with tap water to remove any attached eggs. The number of intact eggs in the rinse water was counted in an enamel pan; egg loss was assumed to be due to predation. Predation was verified by the presence of macerated egg chorion in the vial or in the animal's feces (determined by bleaching and microscopic examination - see Ch. 3). Up to 10 replicates per species were preformed. Animals were then placed in 70% isopropyl alcohol before subsequent identification to species level (where possible).

When possible, ants were colonized and the entire colony exposed to mosquito eggs and other food items. Ants were provided water, dilute honey and a variety of items (dead insects, tuna, peanut butter) for food. A colony of carpenter ants (Camponotus abdominalis floridanus (Buckley)) was maintained in the original branch while a colony of Paratrechina bourbonica (Forel) was moved from PVC pipe into a plastic crisper pan (11 X 23.5 X 31 cm) for

colonization. A plastic petri dish (9-cm diameter) was placed in the pan to house the colony.

The petri dish was prepared for colonization in the following manner. The bottom was filled with ca. 1 cm of plaster of Paris that was moistened periodically to maintain high relative humidity. Two small entrance holes were cut in the lid and side of the dish and a cardboard cover was taped to the lid to provide shade. The sides of the crisper pan were painted with Fluon® to prevent ant escape. Colonies were maintained for at least one week before testing.

The following procedure was used to expose ants to Ae. taeniorhynchus eggs. Ten mosquito eggs were placed on 3-cm diameter vial caps (from 25-ml plastic vials) containing a layer of moistened plaster of Paris to prevent desiccation of eggs. Four caps were placed in the bucket or pan housing the colony; the number of eggs missing (presumedly eaten by ants) was tallied after 24 hr. Observations of feeding behavior were made during the course of the experiment. The mean percentage of eggs missing was calculated from 6 replicates. In one trial, the C. abdominalis floridanus colony was exposed to four red mangrove leaves with Ae. taeniorhynchus eggs attached by silicone caulk. The egg loss per leaf was determined after 24 hr.

Food preference studies were also conducted. The ants were offered four similarly prepared vial caps, two containing

mosquito eggs, and two containing an animal selected for its approximation to potential prey found in the mangrove swamp. Living animals offered were 4th instar Ae. taeniorhynchus larvae and adult coffee snails ( Melampus coffeus Linne). Freshly killed animals offered were adult female Ae. taeniorhynchus, adult M. coffeus, 3rd instar cabbage loopers (Trichoplusia ni (Hubner)) and mature mosquitofish (Gambusia affinis Girard). Responses of ants were observed for at least 1/2 hour following introduction of food. Preference was assessed qualitatively by (1) speed of arrival at an item, (2) number of ants at an item and, most significantly, (3) ingestion and/or transport of an item to the colony. Typically, preferred items were "attacked" quickly and taken to the petri dish whereas nonpreferred items, despite inspection, were not eaten or removed.

Crabs were exposed to Ae. taeniorhynchus eggs in 10-liter plastic buckets. Upland soil from Dogwood, previously frozen to kill predators and resident mosquito eggs, was used to line the bottom of the bucket. Four 5-cm diameter PVC rings were pushed into the soil, the top positioned flush with the surface. Mangrove leaf litter (also previously frozen) was placed on the surface to create realistic cover and the bucket top ringed with vaseline to prevent escape of crabs. One crab (Sesarma ricordi H. Milne Edwards) was placed in the bucket and

allowed to acclimate for 24 hr before 20 mosquito eggs were placed within each ring. After 24 hr, the crab was removed and preserved in alcohol for later identification. The rings and accompanying soil were removed and the rings and bucket were flooded separately with yeast infusion to hatch mosquito eggs; larvae were counted 24 hrs later. Appearance of larvae in the bucket suggested that eggs were transported incidentally by the crab. Three control buckets containing mosquito eggs but no crabs were processed similarly. The fiddler crab Uca rapax (Smith) was tested similarly except that eggs glued to red mangrove leaves (two leaves per test) with silicone caulk were used. Egg predation was estimated by the number of missing eggs.

Preliminary studies with the coffee snail M. coffeus indicated that many mosquito eggs passed through the snail's digestive tract with no noticeable damage. Thus, an experiment was conducted to establish the proportion of snail-ingested eggs passed unscathed, and of these, the proportion that could hatch. Snails were placed in a 25-ml plastic vial with a large number of Ae. taeniorhynchus eggs and a piece of red mangrove detritus. After 24 hr, the snails were rinsed in distilled water to remove uningested eggs and then were placed in a clean vial; red mangrove detritus was provided for food. Snail feces were removed and placed in a 25-ml plastic vial that was then incubated at least 48 hrs before flooding with yeast infusion in an

attempt to hatch mosquito eggs. Larvae were counted and the feces were bleached in 5% sodium hypochlorite to facilitate egg collecting (see Ch. 3). Eggs were categorized as (1) unhatched and viable (determined by bleaching and observing embryo), (2) crushed with no embryo noted (presumedly infertile), (3) hatched and no larvae present (larva presumedly escaped), and (4) hatched with dead larvae present. The latter category indicates that eggs can hatch within the snail's digestive tract but that the larvae are killed.

The large populations of M. coffeus noted at the April study site (Ch. 2) suggested that its role as a predator of mosquito eggs should be tested under more realistic conditions. Ten-cm diameter mangrove sods were collected from an area known to have mosquito egg and snail populations. The sods were tightly sealed in plastic dishes (11 cm diameter, 8 cm height) and then frozen at least 24 hr to kill predators and resident eggs. One hundred Ae. taeniorhynchus eggs (from field-collected females) and two field-collected M. coffeus were added to each sod; the lid was replaced and the sod was incubated at room temperature for one week. Snails were removed from the sod before it was flooded with yeast infusion; the mosquito larvae were counted after 24 hr. Two trials, consisting of seven and eight test replicates and six control replicates (similarly prepared sods except no snails were added) were conducted.

## Results

### Field Estimates of Egg Mortality

Two methods were developed to quantify egg loss in fine-grained and detrital soil. The use of silicone caulk to fix eggs to a substrate for field placement (leaf test) had several advantages over simply placing eggs upon the substrate (ring test). A direct count of egg loss could be easily and accurately conducted; no correction factors for sampling efficiency, resident eggs or recent oviposition are needed. The egg-bearing leaves could be placed quickly at any location; this method was used to place eggs underwater to study the senescence of submerged eggs (see Ch. 6). Finally, weekly predation and senescence rate was derived by subtracting the number of missing and dead eggs from the original number of eggs on the leaf. The ring test, on the other hand, was tedious to prepare, process and analyze. The ring test was also subject to several sources of error including egg hatching, larval survival, egg sampling, resident eggs and recent oviposition. Unfortunately, it may be the most realistic method to estimate egg loss from fine-grained soil. Perhaps silicone caulk can be adapted for this situation.

Upland and lowland egg mortality rate both declined during cooler weather (Fig. 5-1). This reflects the impact of lower temperatures on predator activity. The higher loss rates for lowland vs upland sites during the summer

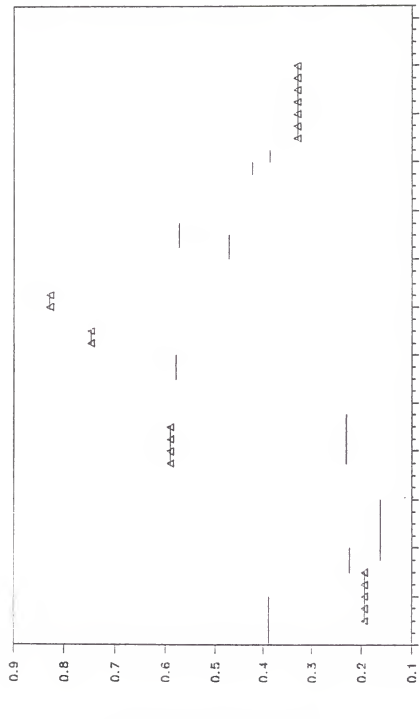



Figure 5-1. A figure depicting egg mortality rates for upland (line) and lowland (connected symbols) at Dogwood. Length and position of line represent exposure period and date, respectively, for eggs placed in the field.

may be experimental artifacts. Egg-bearing leaves were placed at the top of the compacted peat layer and might be more exposed to predation than eggs placed in the soil. Aedes taeniorhynchus were found to oviposit several layers below the top peat layer (see Ch. 2), perhaps to minimize exposure of eggs to predation as well as desiccation.

Results for the vial test are presented in Table 5-1 through Table 5-3. Data are presented by taxa; a list of all animals tested is provided in Appendix D. Results represent mean percentage of eggs lost (presumedly eaten). The presence of macerated egg chorion in the vial or in the test animal's feces was positive evidence for egg predation.

The vial test indicated that several noninsect inverterbates are potential mosquito egg predators. Unfortunately, no data on species abundance are available; comments referring to abundance are subjectively based on personal observations. The amphipod Talitrus specificus (Hurley) ingested eggs readily and was very abundant in litter at both study sites; this species may be a dominant predator of Ae. taeniorhynchus eggs in mangrove. The isopods Porcellio virgatus (Budde-Lund) and Tylos niveus Budde-Lund also consumed mosquito eggs readily; P. virgatus was common in some mangrove forests. The one millipede (Diplopoda) collected did ingest eggs but is probably not a significant predator due to its scarcity. Two of the three

snails tested ingested mosquito eggs (results for M. coffeus will be presented later). Ellobium pellucens (Menke) ingested eggs, most of which were found crushed within the feces. This snail was found commonly at Dogwood, albeit in low numbers. Polygyra cereolus (Mulhlfeldt), collected only once, is probably not a significant predator of eggs.

Table 5-1. Mean percentage of eggs (n = 20) presumedly consumed by the following invertebrates (excluding insects) during 24-hr exposure in a 25-ml vial. Adult animals tested unless noted.

| Test animal | Reps. | % consumed | Evidence[a] |
|---|---|---|---|
| A. Order Amphipoda | | | |
| 1. Talitrus specificus | (13) | 50.4 | + |
| B. Order Diplopoda | | | |
| 1. unidentified | ( 1) | 65.0[b] | |
| C. Order Isopoda | | | |
| 1. Porcellio virgatus | (10) | 65.5 | + |
| 2. Tylos niveus | ( 9) | 21.1 | + |
| D. Phylum Mollusca | | | |
| 1. Ellobium pellucens | ( 6) | 36.7 | + |
| 2. Polygyra coreolus | ( 1) | 20.0 | - |

[a] Macerated egg chorion present in vial or feces

[b] Eggs exposed to animal for 72 hr

Several species of insects ingested mosquito eggs in the vial test (Table 5-2; classification based on Borror et

Table 5-2. Mean percentage of eggs (n = 20) presumedly consumed by the following insects during 24-hr exposure in a 25-ml vial. Adults tested except where noted.

| Test insect | Reps. | % consumed | Evidence[a] |
|---|---|---|---|
| Order Collembola | | | |
| 1. unidentified[b] | ( 6) | 0.8 | - |
| Order Orthoptera | | | |
| Family Gryllidae | | | |
| 1. Neonemobius cubensis | ( 5) | 96.0 | + |
| Family Blattidae | | | |
| 1. unidentified | (10) | 18.0 | + |
| Order Coleoptera | | | |
| Family Carabidae | | | |
| 1. Loxandrus sp. (larva) | ( 2) | 25.0 | + |
| 2. Loxandrus rectangulus | (10) | 20.5 | + |
| 3. Oodes amaroides | ( 4) | 20.0 | + |
| 4. Scarites sp. (larva) | ( 1) | 30.0 | - |
| Family Staphylinidae | | | |
| 1. Achenomorphus corticinus | ( 1) | 10.0 | + |
| 2. Acylophorus princeps | ( 1) | 5.0 | - |
| 3. Acylophorus sp. | ( 5) | 1.0 | + |
| 4. Astenus sp. | ( 1) | 5.0 | - |
| 5. Carpelimus maculicollis | ( 3) | 8.3 | + |
| 6. Carpelimus sp. | ( 7) | 2.9 | - |
| 7. Manda nearctica | ( 1) | 15.0 | - |
| 8. Myllaena insipiens | ( 2) | 12.5 | + |
| 9. Neobisnius ludicrus | ( 1) | 20.0 | - |
| 10. Neohypnus pusillus | ( 1) | 100.0 | + |
| 11. Philonthus alumnus | ( 2) | 10.0 | + |
| 12. Pinophilus sp. (larva) | ( 1) | 10.0 | - |
| 13. Pinophilus sp. | ( 4) | 30.0 | + |
| 14. Scopaeus elaboratus | ( 1) | 10.0 | + |
| 15. Scopaeus sp. | ( 5) | 37.0 | + |
| 16. Stamnoderus pallidus | ( 1) | 5.0 | - |
| 17. Subfamily Paederinae (larva) | ( 1) | 20.0 | + |

Table 5-2 continued

| Test insect | Reps. | % consumed | Evidence[a] |
|---|---|---|---|
| Order Hymenoptera | | | |
| Family Formicidae | | | |
| 1. Hyponera opaciceps[b] | (18) | 47.5 | + |

[a] macerated egg chorion present in vial or in feces

[b] two test animals in a vial

al. 1976). The cricket Neonomobius cubensis (Saussure) readily ate mosquito eggs; however, its scarcity suggests that its role as an egg predator is minimal. Insects that readily ingested eggs in the vial and were commonly observed or collected in the field included the carabid Loxandrus rectangulus LeConte, staphylinids as a group, and the formicid Hyponera opaciceps (Mayr).

Results from predation studies suggest that some ants may be significant predators of Ae. taeniorhynchus eggs. The ant H. opaciceps, although not colonized, readily ingested mosquito eggs in vials. Colonies of C. abdominalis floridanus and P. bourbonica were established long enough to expose to mosquito eggs. The results suggest that P. bourbonica may be a significant mosquito egg predator; it ate mosquito eggs presented on the plaster-filled lids (62.1% eaten in 24 hrs exposure tests, n = 6). For two

trial using eggs placed on plastic lids, C. abdominalis floridanus ate 24.2% of the eggs. Only 6.6% of the eggs that were glued to leaves were lost when exposed to this ant for 24 hr.

Preference studies indicate that the ants tested preferred larger prey items to mosquito eggs (if the item is palatable). Table 5-3 summarizes feeding preference trials with P. bourbonica. This ant preferred mosquito larvae, freshly killed cabbage loopers and adult mosquitoes to mosquito eggs. Large prey items such as mosquito larvae and adults certainly are a larger cache for ants, and thus might be preferred over mosquito eggs. Also, freshly killed prey might provide both more attractive and more voluminous olfactory cues to the ants than might "inert" mosquito eggs. The ants showed interest in neither the snails nor the mosquitofish. This may explain why large populations of M. coffeus occur in mangroves; perhaps they are chemically protected from ant predation. The same may be true for recently stranded mosquitofish; even limited protection from ants might enable fish to survive for a short period. Ants (not identified) were observed feeding on dead mosquitofish in the field. Observations on the ant C. abdominalis floridanus were limited; in the only experiment conducted before the colony escaped, the ants did seem to prefer larger dead insects (freshly killed adult noctuids) to mosquito eggs.

Table 5-3. The preference of the ant Paratrechina bourbonica for Aedes taeniorhynchus eggs presented simultaneously with the alternative food items. Dead prey were freshly killed.

| Alternative item | Preference for |
|---|---|
| Mosquito (Aedes taeniorhynchus) | |
| Live 4th instar larvae | larvae |
| Dead adult females | adult females |
| Snail (Melampus coffeus) | |
| live adult | mosquito eggs |
| dead adult | mosquito eggs |
| Cabbage looper (Trichoplusia ni) | |
| dead 3rd instar larvae | cabbage looper |
| Mosquitofish (Gambusia affinis) | |
| dead adult | mosquito eggs |

Neither crab tested (S. ricordi and U. rapax) appeared to be a significant predator of mosquito eggs. The number (mean ± SD) of larvae hatching after 48 hrs exposure to S. ricordi was 15.1 ± 3.4 for soil within PVC rings and 3.6 ± 3.5 for soil outside the rings. For the three controls, a mean of 16.7 ± 3.7 and 0.7 ± 1.2 larvae hatched from soil collected within and outside the rings, respectively. The results suggest that little egg predation occurred, although crabs may transport eggs incidentally; in one test, 11 larvae (37% of all larvae hatched in test) were found in soil outside the rings. Results from the tests with U. rapax were as follows: 30.0 ± 18.3 eggs were removed (possibly eaten) from leaves during the 48 hrs exposure period.

These data suggest that adult U. rapax and S. ricordi are only incidental predators of mosquito eggs. If mosquito eggs are encountered by grazing crabs, predation and even incidental transport may occur; predation is apparently a function of the relative abundance of mosquito eggs and crabs. Perhaps immature crabs, commonly present in greater densities, may be more effective predators of mosquito eggs.

Studies with the snail M. coffeus indicate that mosquito eggs can survive despite ingestion. Figure 5-2 shows the fate of eggs exposed to a hatching stimulus following passage through the digestive tract of M. coffeus. The greatest percentage of eggs remains unhatched (56.9%), followed by gut-hatched (20.0%), successfully hatched (10.6%) and crushed eggs (3.5%). The latter are probably infertile as infertility rates of field-collected eggs are similarly below 10% (Ch. 6). The hatching of eggs within the feces may be caused by flooding with the yeast infusion or by conditions within the snail's gastrointestinal tract.

Despite the willingness of M. coffeus to ingest Ae. taeniorhynchus eggs, this snail appears to be a relatively ineffective mosquito egg predator. Mortality of mosquito eggs exposed to snails within a dish containing mangrove soil was insignificant (Table 5-4). The coffee snail, like the crabs, appears to be an incidental predator of mosquito eggs where predation is a matter of chance encounter.

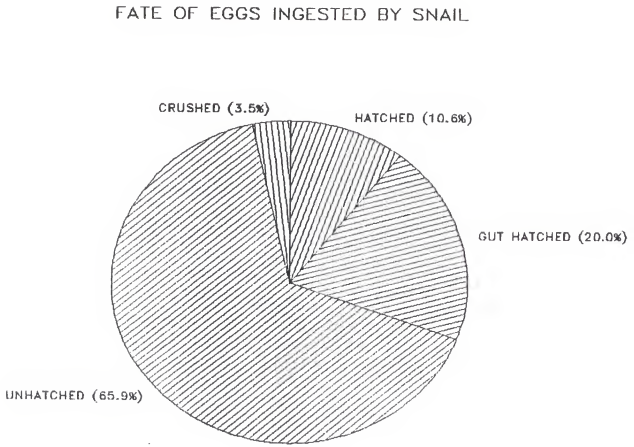



Figure 5-2. Diagram showing the fate of Aedes taeniorhynchus eggs ingested and excreted by the pulmonate snail, Melampus coffeus. Snail feces were incubated for 48-hr then flooded with a yeast infusion to induce mosquito egg hatching (see text for description of egg categories).

Table 5-4. Percent of Aedes taeniorhynchus eggs (100 original eggs) hatching from 10-cm diameter sod after 1 week exposure to 2 Melampus coffeus. Values are mean ± SD.

| Trial | Test | Control |
|---|---|---|
| A | 81.1 ± 10.1 (n=8) | 89.0 ± 6.5 (n=3) |
| B | 52.7 ± 21.8 (n=7) | 64.7 ± 25.6 (n=3) |

## Discussion

Methods were successfully developed to quantify egg mortality for fine-grained and detrital soil. The use of PVC rings and the plexiglass cover provided a way to measure egg mortality in compacted fine-grained soil. The silicone caulk provided a way to quantify egg predation in detrital substrates without using a cover. The latter method is easier to conduct, is less subject to sampling error, and provides for simpler calculations. Laboratory observations with the snail M. coffeus, coupled with the high predation rates obtained in the field suggest silicone caulk is not repellent to mosquito egg predators, although definitive studies are needed to validate this method.

The data suggest that predation rather than senescence contributes most to egg mortality. Aedes taeniorhynchus eggs subjected to low predation rates can live several months (Bidlingmayer 1956). Focks et al. (1988a) used a value of 0.9985 for the daily survival of

nondiapausing Psorophora columbiae eggs in a Louisiana rice field; this represents a weekly egg loss rate of only 1%. This value, estimated from data by Olson and Meek (1979), strictly involves senescence. The discrepancy between egg survival rates determined by Olson and Meek (1979) and values obtained in this study suggests that egg predation has a great impact on egg survival and mosquito population dynamics.

The seasonal fluctuations in egg mortality supports similar findings for this mosquito by J. H. Frank in Vero Beach, Florida (unpublished data) and Bidlingmayer and Schoof (1956) in Savannah, Georgia. Obviously, warm weather would increase invertebrate activity and, probably, mosquito egg predation as well. Additionally, higher water tables would concentrate terrestrial predators at high elevations of the mangrove forest, increasing the likelihood that an egg would be exposed to a predator.

Nonetheless, the high egg predation rates from the leaf test suggest that this method may overestimate predation rates. The test eggs (glued to the leaf) may be more exposed to predators than eggs deposited naturally; Ae. taeniorhynchus eggs have been observed within broken twigs and in the exposed midrib and petiole of dead red mangrove leaves (S. A. Ritchie, unpublished data). Selective oviposition, coupled with a clumped distribution (see Ch. 2), could provide egg refugia that minimize the probability of

local extinction despite high predation pressure. Refugia (Hassell 1978) and clumped distributions (Murdoch and Oaten 1975) produce stability in prey populations. Additional field study is necessary to validate the presence of egg refugia for Ae. taeniorhynchus.

Density-dependent egg predation could also produce population stability (Service 1985). However, a response lag between prey and predator populations can result in extreme fluctuations in prey populations (May et al. 1974). Perhaps this contributes to the explosive outbreaks of Ae. taeniorhynchus. Extremely high mosquito egg densities might saturate the egg predators, resulting in reduced egg predation; the influx of migratory adult mosquitoes would be likely to exacerbate the situation. If predation is density-dependent, perhaps more efficient mosquito control can be achieved by maintenance of egg populations at or below levels that can be controlled by native egg predators. Perhaps biocontrol agents could be introduced, reared, or augmented to help maintain mosquito egg populations at acceptable levels. Amphipods, isopods, ants and beetles appear to be the best candidates.

# CHAPTER 6
# CONSTRUCTION AND SENSITIVITY ANALYSIS OF A SIMULATION MODEL OF *AEDES TAENIORHYNCHUS*

## Introduction

Development of a simulation model of *Aedes taeniorhynchus* is based upon the premise that mosquito production is driven by a dynamic and predictable interaction of the biotic and abiotic factors present in the mosquitoes' environment. Through the use of Forrester's (1961) feedback dynamics method, a hypothesis delineating the most significant factors will be examined. The biotic factors include reproductive capacity and predator population levels; the abiotic factors are temperature, rainfall, tide and water level of the mosquito-producing habitat, i.e. the mangrove basin forest.

The relationships of these factors are complex and necessitate computer simulation to demonstrate their impact on mosquito population dynamics. Mosquito and hydrological models were independently constructed, then coupled to produce the final simulation model. A computer spreadsheet (Hancock and Heaney 1987, Rowan et al. 1988) was used to develop a model to simulate Dogwood water levels. This chapter, however, will only concern the construction and sensitivity analysis of the *Ae. taeniorhynchus* population

model (TAENISIM). TAENISIM will be coupled with the water level model in Ch. 8.

The model was constructed using the feedback dynamics approach of Forrester (1961) and Roberts et al. (1983a). Feedback dynamics has proved to be an excellent way to investigate complex system dynamics associated with ecological systems (Montague et al. 1982). Hypothetical causal mechanisms of mosquito population dynamics were formalized as feedback loops; positive feedback loops account for population growth while negative feedback loops limit population growth. The feedback loop model was used to develop a flow diagram (Forrester 1961) representing the impact of the feedback loop parameters on the population dynamics of each stage of the mosquito life cycle. This impact was expressed in terms of changes in the rate of flow of individuals in and out of specific life stages (termed level); e.g. temperature affects the rate of flow of eggs into larvae due to changes in the hatching percentage. The interaction of parameters delineated in the feedback loop diagram and flow rates for population levels were written as mathematical equations (rate equations) derived from data gleaned from the literature, by experimentation or by personal communication with an authority on the subject. At times, data were limited and an estimate was made. Most experimental work conducted in this study involved the egg stage due to a dearth of published data.

The rate equations were then translated into computer language and assembled to complete the model. All computer statements were written in QUICKBASIC® assembled for use by the model simulation program SYSDYN (Roberts et al. 1983b). Sensitivity analysis was then performed to determine the relative change in model output (e.g. number of eggs in late May) due to fixed changes in rate parameters.

The finished model will be an asset to mosquito research and control. A valid model can be used to identify and test theories of mosquito population dynamics and to identify scenarios associated with prescribed population changes. For example, the model could be used to test the theories of Ritchie (1983) concerning the Ae. taeniorhynchus population crash of 1983. Additionally, the model can be used to examine and optimize the efficacy of control strategies and surveillance (Haile and Weidhaas 1977). The next chapter will detail validation and application of TAENISIM.

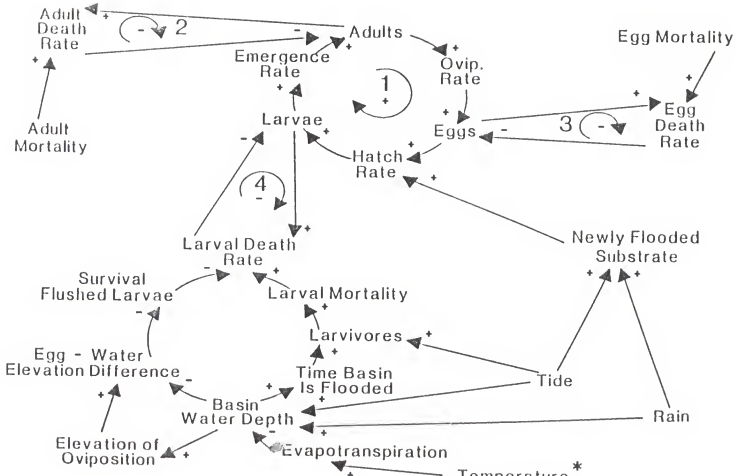
## Materials and Methods; Results

These sections are combined to enhance reader comprehension of the formulation of TAENISIM. The rate equations are best understood if data collection methods and subsequent equation formulation are presented simultaneously.

Model conceptualization

A flow diagram was developed to summarize a hypothetical description of the Ae. taeniorhynchus life cycle (Fig. 6-1). One positive and three negative feedback loops were hypothesized to account for the population dynamics of Ae. taeniorhynchus in the Dogwood basin. The life cycle loop (loop 1) is a positive feedback loop responsible for population growth while the stage-specific mortality loops (loops 2 to 4) control population growth. The stage-specific mortality loops are affected by several auxiliaries. Hydrological auxiliaries play a significant role in increasing larval mortality by affecting the survival of flushed larvae (i.e. runoff hatched larvae) and by increasing larvivore populations. Hydrology also affects the life-cycle loop since hatching increases as the area of newly flooded substrate increases. Temperature, although not depicted in any loops, affects all life-cycle rates directly.

Three factors thought to be significant factors in Ae. taeniorhynchus population dynamics are not included in the model. Adult migration and mortality due to pesticides are not incorporated in TAENISIM, although later model versions will do so. Most importantly, basin inundation, considered to be an effective mechanism to prevent oviposition and control mosquito production (Clements and Rodgers 1963, Provost 1977), had no effect on oviposition in TAENISIM.




* Temperature also affects all life cycle rates

Figure 6-1. A causal loop diagram of hypothetical relationships in the mangrove basin forest-Aedes taeniorhynchus system used to develope TAENISIM (see text for a discussion of diagram).

Egg surveys (Ch. 3) indicate that mosquitoes continue to oviposit on exposed soil when the basin is fully flooded.

Model construction

Model construction involved translating the hypothesis (Fig. 6-1) into equations comprising TAENISIM. Equations were developed for levels, rates and auxiliaries. Levels refer to the type of population being modeled, such as mosquito eggs, larvae and adults. Rates are the numeric flow of individuals to and from a level; e.g. hatching, emergence and death rates. Auxiliaries are parameters such as temperature and rainfall, that are outside the populations being modeled but that affect rates. This section discusses this process for each equation type used in the model.

Level equations

Three basic population levels are used in TAENISIM. Populations for eggs, larva-pupa and adult females are calculated using level equations. Larvae and pupae are combined since they occupy the same habitat sequentially. Adult male populations are not used in the model because they are not pestiferous. Mating success of the females is incorporated in the fertility rate of eggs. Egg levels are stratified to follow discrete populations of immature and submerged eggs that do not hatch.

Some level equations are divided to estimate populations separated by elevation and time. Egg levels are placed into eight sublevels (strata) according to the elevation of the soil containing the eggs. Since Dogwood had a flooding range of 1.2 - 2.8 ft above mean sea level (MSL), eight strata with a resolution of 0.2 ft are used (Table 6-1); measurements are in feet to correspond to hydrological data obtained from the United States Geological Survey. This allowed oviposition and hatching to be followed relative to changes in the water table. The elevations chosen correspond to elevations where eggs historically have been found (see Ch. 2).

Table 6-1. Criteria for egg-level stratification used in model construction.

| Level stratum | Elevation range (ft. above MSL) |
|---|---|
| 1 | < 1.6 |
| 2 | 1.6+ - 1.8 |
| 3 | 1.8+ - 2.0 |
| 4 | 2.0+ - 2.2 |
| 5 | 2.2+ - 2.4 |
| 6 | 2.4+ - 2.6 |
| 7 | 2.6+ - 2.8 |
| 8 | 2.8+ |

Larval and adult female populations also are stratified into time cohorts. Three strata are used for larval levels so that overlapping broods can be followed discretely. Otherwise, larvae hatching three days later would

be placed in the same level as the initial brood, and thus appear to emerge three days prematurely. Three strata were chosen because it is unlikely that more than three larval broods will be present at any one time. A similar problem concerning oviposition timing for overlapping adult female populations was treated in the same manner.

Rate equations.

Rate equations are created for egg, larval and adult levels. The data source, experimental method, results and rate equation are provided. All variable names are written in capital letters; 'T' refers to the time of year expressed in weeks. Rates are in terms of proportional change per week for each population level input. Most rates are either divided by 0.01 or raised to the 0.01 power because 0.01 week is the model time-step. Time parameters used in TAENISIM are 'T', the unit time of 1 week ; 'T0', the computational time step; 'T1', the run starting time; 'T2', the run stop time and 'T3'; the plotting interval. Modified sine functions are used to describe many rates affected by seasonal temperature changes; Fig. 6-2 indicates the sine wave relationship of mean daily temperature to time of year for Naples, FL. LOTUS 123® was used to fit equations to data and to plot the function values. IF statements are used to limit values when rate equations

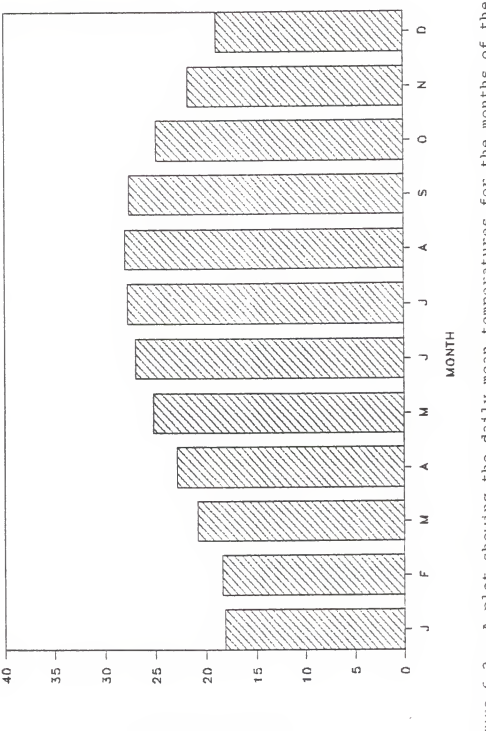



Figure 6-2. A plot showing the daily mean temperatures for the months of the year in Naples, Florida (data from 1960 to 1985).

provide unrealistic values. A complete program listing is provided in Appendix E.

Eggs. Several rates are determined for egg levels. Much of the information was derived from field experiments conducted at Dogwood or from laboratory experiments using mosquitoes collected at Dogwood.

Oviposition. Estimation of oviposition rates involved several auxiliaries and constants. Generally, oviposition represents the product of gravid mosquitoes and clutch size. The frequency of oviposition, i.e. length of the gonotrophic cycle, varies proportionally with temperature. This period involves time spent obtaining a bloodmeal, developing eggs and ovipositing. Edman (1985) reports a preoviposition period of 3 to 5 days for Ae. taeniorhynchus in Florida; this probably represents a summer value. Winter values are probably longer; Fig. 6-3 shows the value of estimated gonotrophic cycle calculated with Eq. 6-1.

PREOVIPOSTIME = 1.1 + (0.9 X sine(2Pi X (T+10)/52)) (6-1).

Summer rates are limited to no less than 0.6 week by an IF statement. A timer (GRAVTIME) is set for each brood; when GRAVTIME is equal or greater than PREOVIPOSTIME, oviposition occurred. GRAVTIME is set back two days (2/7 T) at adult emergence to allow for migration, mating and

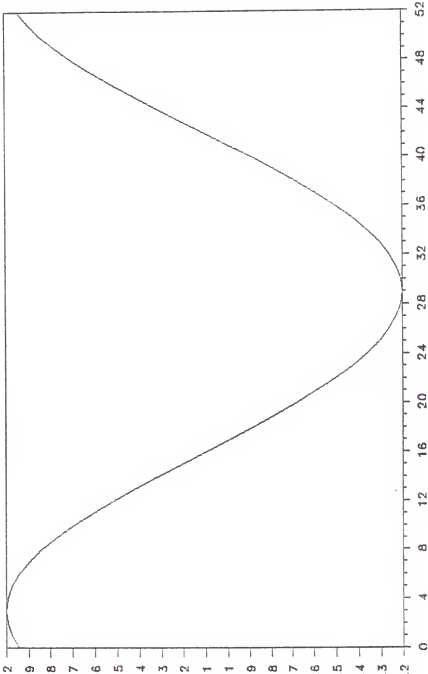



Figure 6-3. A plot showing preoviposition time (yearly values) used in TAENISIM.

bloodfeeding (Edman 1985, Provost 1962). Clutch size was estimated for autogenous and anautogenous mosquitoes. An autogenously produced (produced without a blood meal) clutch is estimated as a constant 25 eggs per gonotrophic cycle (O'Meara, personal communication). The clutch size for anautogenously produced eggs (produced following a blood meal) was determined by bloodfeeding Dogwood mosquitoes on a mouse then counting the eggs deposited in a vial containing mangrove peat. The mean clutch size for anautogenously produced eggs was 81.8. Fertility was determined by bleaching eggs gathered from five broods collected at Dogwood. Data for clutch size, fertility and hatch for Dogwood Ae. taeniorhynchus is shown in Table 6-2. Viable egg production is calculated by multiplying clutch size by fertility (0.914).

The proportions of eggs produced autogenously and anautogenously are estimated. O'Meara (personal communication) suggested that 85% of south Florida Ae. taeniorhynchus are autogenous for the first gonotrophic cycle. Thus, the proportion of anautogenously (BFED) and autogenously (AUTOGEN) produced eggs is written in TAENISIM as 1.) BFED = 0.15, and 2.) AUTOGEN = 1 - BFED for the initial gonotrophic cycle. Gonotrophic cycles are tracked by GONOCYCLE. Further oviposition (GONOCYCLE > 1) involved resetting the proportion of autogenous and anautogenous mosquitoes to 0 and 0.80, respectively.

Table 6-2. Hatching percentage, clutch size and fertility of eggs from field-collected Aedes taeniorhynchus. Parenthetical values represent mean temperature (C) in cooler prior to flooding and of water used to flood, respectively. NA represents data not available. Mean hatching percentages followed by different letters are significantly different at the 0.05 level.

| Date collected, hatched | N | Mean | SE |
|---|---|---|---|
| 1. 30 May, 17 June | | | |
| % hatch (27, 22) | 30 | 98.9,A | 0.5 |
| clutch size | 30 | 104.8 | 6.0 |
| % fertile | 30 | 98.7 | 0.7 |
| 2. 21 Aug., 14 Sept. | | | |
| % hatch (27, 22) | 30 | 95.3,A | 1.5 |
| clutch size | 30 | 58.3 | 3.4 |
| % fertile | 30 | 98.1 | 1.0 |
| 3. 12 Oct., 26 Oct. | | | |
| % hatch (20, 19) | 27 | 53.8,B | 6.7 |
| clutch size | 27 | 82.3 | 7.6 |
| % fertile | 27 | 92.3 | 0.4 |
| 4. 22 Nov., 13 Dec. | | | |
| % hatch (17, 23) | 33 | 55.2,B | 6.6 |
| % hatch (18, 13) | 15 | 9.0,C | 5.9 |
| clutch size | 33 | 93.7 | 7.8 |
| % fertile | 15 | 92.5 | NA |
| 5. 1 Feb., 2 Mar. | | | |
| % hatch (18, 18) | 30 | 94.8,A | 0.9 |
| clutch size | 31 | 69.9 | 3.2 |
| % fertile | 31 | 96.6 | 0.7 |
| 6. 26 March, 13 April | | | |
| % hatch | NA | 93.8 | NA |

O'Meara (personal communication) suggested that autogeny is not expressed after the initial oviposition. However, it is felt 80% rather than 100% more realistically represented the proportion of anautogenous females that would produce an entire clutch for subsequent gonotrophic cycles.

Total oviposition is the product of number of adult females, proportion bloodfed and clutch size. Oviposition is stratified (i = stratum) for up to three overlapping broods (Eq. 6-2).

$$OVIP(i) = (ADULTFEM(i) \times BFED \times EGGBATCH) + (ADULTFEM(i) \times AUTOGEN \times AUTOEGG))/T0 \quad (6\text{-}2).$$

Egg distribution. Aedine mosquitoes are known to oviposit preferentially in response to soil moisture (Knight and Baker 1962, Meek and Williams 1986). Egg surveillance at Dogwood suggests that Ae. taeniorhynchus oviposits preferentially within 0.2 - 0.6 ft MSL above the waterlevel. Therefore, the cumulative distribution of eggs vs elevation is a log function that appears to steepen as the water level increases. A sigmoid equation was fitted to these data (Fig. 6-4); the estimated cumulative egg distribution was not significantly different ($P > 0.50$ for F test (Schlotzhauer and Littell 1987)) from the field data for the three hydrological conditions shown in Fig. 6-4. This equation can be used to estimate oviposition for any stratum under all watertable conditions. In TAENISIM, egg

distribution is defined by the cumulative percentage of oviposition occurring at each stratum. This value (POVIPOS(i)) is determined by the current watertable (NEWTABLE) and stratum elevation (ELEV(i)) for i = 1 to 8 strata (Eq. 6-3).

$$POVIPOS(i) = 100/(1 + (0.02/(NEWTABLE/ELEV(i)) \times (ELEV(i) - (NEWTABLE + 0.10))^{-3}) \quad (6\text{-}3).$$

Zero values are removed by an IF statement and the cumulative percentage of oviposition set to 100 (calculated POVIPOS(8) may be slightly less than 100) for stratum 8. The percent of oviposition for a stratum is calculated by multiplying total oviposition by the difference in cumulative percent oviposition for the ith and i - 1 strata (Eq. 6-4).

$$OVIPOS(i) = ((POVIPOS(i) - POVIPOS(i-1))/100) \times OVIPOS \quad (6\text{-}4).$$

Because elevation strata vary in size, the number of eggs oviposited per stratum is calculated by multiplying OVIPOS(i) by an area correction factor (PAREA(i); Eq. 6-5) calculated from the stage-area relationship derived in Ch. 7.

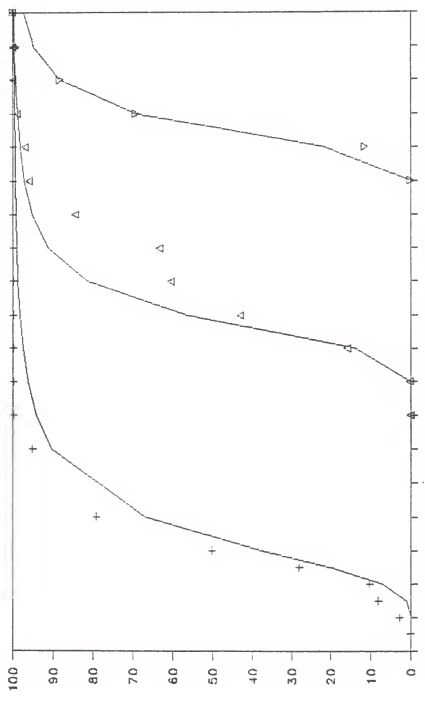



Figure 6-4. A plot of the estimated cumulative distribution of oviposition used in TAENISIM for 3 hydrologic conditions at Dogwood. The solid lines are model output and the symbols are field estimates based on samples of eggs.

PAREA(i) = AREA(i)/((1/8) X TOTALAREA) (6-5);

where AREA(i) is the area of statum i, TOTALAREA is the total area of the basin and 1/8 X TOTALAREA is the area for 8 strata of equal size.

Egg maturation. Egg maturation time (EGGMAT) was estimated from val-ues obtained from Nayar (1985). Eggs mature in three to ten days when incubated at 30 and 20 °C (Nayar 1985), respectively. A sine function is used to simulate seasonal changes in EGGMAT (Eq. 6-6; Fig. 6-5).

EGGMAT = 1.1 + 0.9 X sine(2Pi X (T + 10)/52)) (6-6).

EGGMAT ranged from 3 - 14 days; summer values are limited to values > 3 days by an IF statement. Immature eggs are transferred to mature egg levels when a counter (EGGMATURETIME; initialized at 0 and stepped at T0 increments starting at the moment of oviposition) is greater or equal to EGGMAT.

Maturation of submerged immature eggs was investigated experimentally. Eggs < 24 hr old were submerged in distilled water in 6-ml test tubes and were incubated at 22 °C. Their maturation period was then compared that of to a nonsubmerged control group. Maturation was determined by bleaching the egg for direct observation of the embryo; criteria were the development of eye spots, egg burster and abdominal segmentation. Maturation of > 50% of sampled

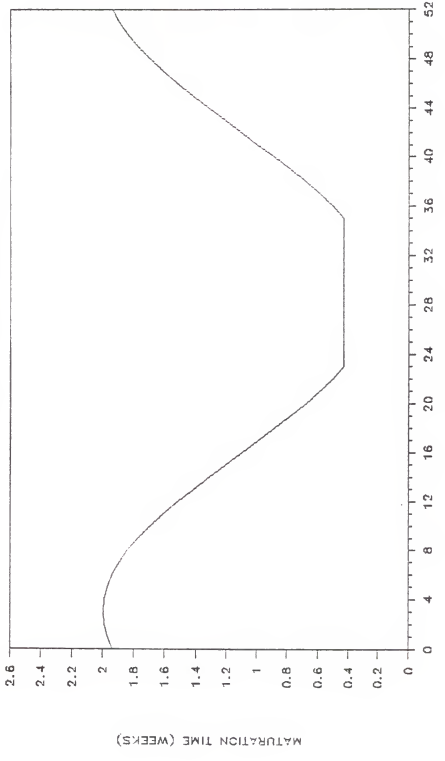



Figure 6-5. A plot of the estimated egg maturation time used in TAENISIM.

eggs was noticed at four and five days post harvest for control and submerged eggs, respectively. These data indicate that submerged eggs do mature, albeit slightly slower than exposed eggs. Because the time difference was minimal, the maturation rate for submerged immature eggs was estimated using EGGMAT.

Egg survival. Egg survival was estimated for exposed (see Ch. 5) and submerged eggs. Because the high egg loss rates at lowland sites eggs during the summer are thought to be higher than natural rates (see Ch. 5), the estimated egg loss (EGGMORT) used in TAENISIM uses the lower upland values during summer. Specifically, EGGMORT = 1 - EGGSURVIVE where EGGSURVIVE is the proportion of eggs surviving/week (Eq. 6-7).

$$EGGSURVIVE = 0.6 + (0.2 \times \sin(2Pi \times (T + 10/52)) \quad (6\text{-}7).$$

EGGMORT and field data are shown in Fig. 6-6. Immature egg mortality is assumed equal to EGGMORT.

The weekly mortality rate for submerged eggs was lower than the rate for exposed eggs. Eggs collected from Dogwood mosquitoes were placed in vials containing 5 ml of distilled water, incubated at 25° C and the proportion of eggs with normal embryos estimated at one, three and five weeks. Survival was 97.8, 92.3 and 97.0% after one, three

and five week submergence, respectively. In a study using brackish water (salinity ca. 10 parts per thousand) collected from Dogwood, the proportion of eggs with normal embryos was 81.9 and 87.7% for two three-week trials and 93.2 and 85.1% for two seven-week trials. Viability was evidenced by hatch (eggs flooded with a yeast infusion) following a 48-hr exposure to ambient air. Clearly, submerged eggs are subject to low rates of senescence in the lab at room temperature.

Field estimates of mortality rates for submerged eggs were considerably higher. In experiments where eggs glued to leaves with silicone caulk were submerged in water, survival was higher than rates estimated for exposed eggs. Submerged egg survival over a two and four week period during August at Dogwood was 82.2 and 16.9%, respectively. These represent weekly mortality rates of 0.093 and 0.359, respectively. In a study using eggs trapped in cheesecloth placed in submerged petri dishes, 39.8% of the recovered eggs (n = 100) had normal-appearing embryos following a seven week exposure (3 Jan. - 21 February 1987), a weekly mortality rate of 0.123. On the basis of this limited data, the mortality rate of submerged eggs (SUBEGGMORT) is a constant value of 0.20 per week.

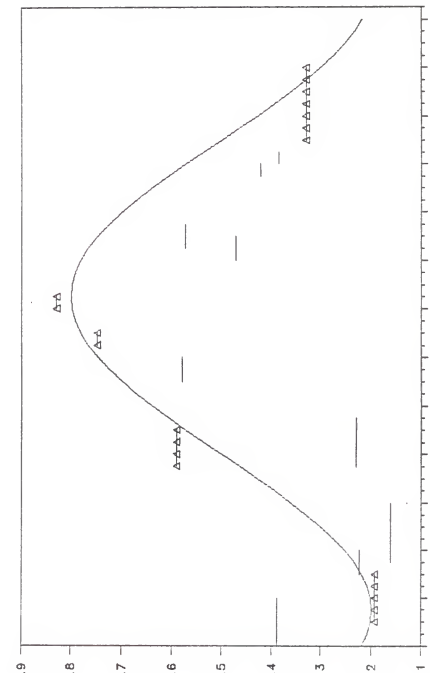



Figure 6-6. A plot of the estimated weekly mortality rate of eggs used in TAENISIM. The solid line is the model output and the symbols are field estimates.

Egg hatching. Hatching percentage was estimated for eggs submerged or exposed to surface runoff. The literature is replete with data concerning the hatching of submerged eggs but no data concerning the hatching success of eggs exposed only to runoff were obtained. The hatching dynamics of such eggs, discussed in detail in Chapter 4, were used to derive the estimates used in TAENISIM.

The hatching dynamics of submerged eggs are well studied. Moore and Bickley (1966) and Parker (1985) found that "winter eggs" of Ae. taeniorhynchus hatched erratically; specifically, hatching percentage decreased at lower temperature and photoperiod. Therefore, experiments were conducted to see if the hatching percentage of Ae. taeniorhynchus eggs collected from Dogwood varied seasonally.

Field-collected mosquitoes were blood-fed on a rodent (mouse or guinea pig) and individually allowed to oviposit on mangrove peat within a 6-ml test tube. Mosquitoes were maintained in a styrofoam cooler with a 10 X 15 cm plexiglass window to allow natural lighting. The cooler was placed in a well-shaded outdoor location. Mosquitoes were given 10% honey water in cotton balls. High relative humidity was maintained with a moist paper towel. A Taylor Max-Min$^{R}$ thermometer recorded the daily temperature range in and out of the cooler and indicated that the cooler decreased the daily temperature range by 2 to 4 $^{o}$C. After incubation in the cooler for ca. two weeks, vials were

flooded with a 1:100,000 (by volume) yeast infusion to induce hatching. Vials were then frozen to kill eggs that might hatch during processing. Hatching success was determined by sieving and bleaching the soil to facilitate rapid counting of hatched and unhatched eggs. Unhatched eggs were cleared to account for unhatchable dead or infertile eggs. The hatching percentage was arcsine transformed and tested for significant differences with the Ryan-Einot-Gabriel-Welsch Multiple F test (Schlotzhauer and Littell 1987); the data and results are presented in in Table 6-2.

The data suggest that hatching percentage is over 90% except during the fall and early winter when cool temperatures reduce this rate. In December, only 55% and 9% of the eggs respectively flooded in 23 and 13 $^{o}$C water hatched while nearly 95% of eggs flooded by 18 $^{o}$C water in early March hatched. These data suggest that reduced hatching is restricted to late fall and winter and is temperature dependent. In TAENISIM, the proportion of egg hatching is estimated by PHATCH (Eq. 6-8).

$$PHATCH = 1.3 + \sin(2\pi \times (T - 11)/52) \qquad (6\text{-}8).$$

PHATCH is limited to values <= 0.95 by an IF statement; Fig. 6-7 shows seasonal values for PHATCH and field data.

The proportion of eggs hatching in response only to runoff only (runoff hatching) is estimated for both lowland and upland substrates of Dogwood. Data from egg surveys

suggest that while runoff hatching occurs in both substrates, hatching is much more extensive for upland substrates. The compacted soil of upland areas is conducive to sheetflow runoff and puddling during heavy rain, whereas runoff trickles readily through the loose detritus of lowland areas, producing neither sheetflow nor puddling. All upland egg populations were less than 2 eggs per sample within 48-hr of heavy rainstorms, while substantial egg populations were found in lowland sites after exposure to heavy rain. Experiments conducted in flumes suggest that eggs adhere tightly to red mangrove detritus but may be flushed from upland soil; nonetheless, the observed velocity of sheetflow at upland sites, coupled with observations of rapid hatching of eggs exposed to runoff, suggest that most eggs are "lost" via runoff hatching rather than via flushing.

These observations, detailed in Chapter 4, are used to estimate the relationship between runoff hatching and rainfall for lowland and upland substrates. In TAENISIM, the proportion of lowland eggs run-off hatching is estimated by PFLHATLO (Eq. 6-9).

PFLHATLO = (RAIN - 0.25) X 0.27 (6-9);

where RAIN = the 24 hr rainfall.

PFLHATLO is limited to values 0 to 0.20 by IF statements.

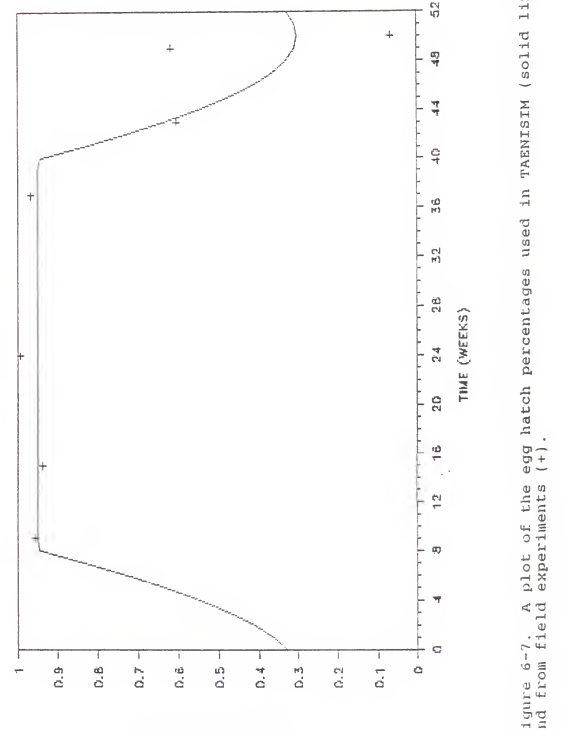



Figure 6-7. A plot of the egg hatch percentages used in TAENISIM (solid line) and from field experiments (+).

The runoff hatch proportion for upland eggs is estimated by PROPFLUSHATCHUP (Eq. 6-10).

PROPFLUSHATCHUP = (RAIN - 0.25) X 1.2 (6-10).

PROPFLUSHATCHUP is limited to a value of 0 - 0.90 by an IF statement. These parameters are plotted in Fig. 6-8.

Larvae/pupae

Survival of flush-hatched larvae. The survival of flush-hatched larvae is also used in TAENISIM and represents the proportion of flush-hatched larvae reaching water that could support complete development. The estimates obtained represent more conjecture based on field and laboratory observation than hard evidence. Mortality of flushed larvae (FLARVMORT) is calculated for each stratum and is dependent upon the substrate and the potential distance to standing water as estimated by the difference between stratum elevation and water level (NEWTABLE). Mortality of flushed larvae (FLARVMORT) for lowland and upland strata is estimated by Eqs. 6-11 and 6-12, respectively.

FLARVMORT(i) = (ELEV(i) - NEWTABLE) X 0.9 (6-11);

FLARVMORT(i) = (ELEV(i) - NEWTABLE) X 0.7 (6-12);

where i = the stratum number and

ELEV(i) = the mean elevation of the ith stratum.

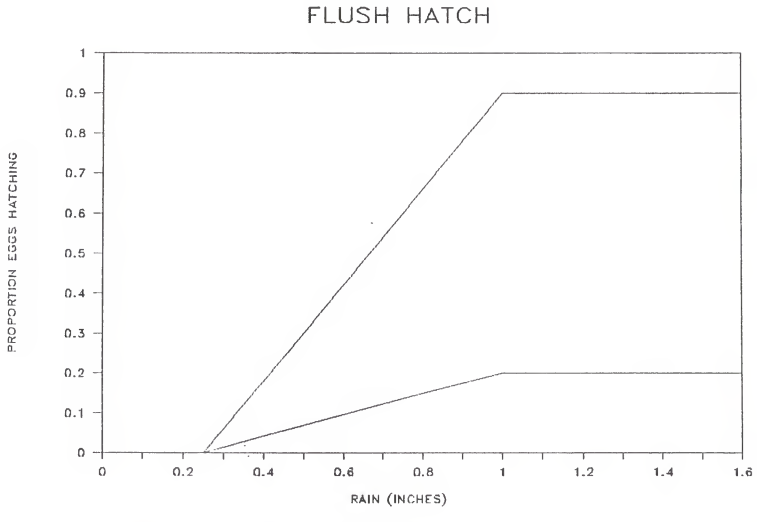



Figure 6-8. A plot of the TAENISIM estimates of the proportion of eggs hatching when exposed to rain-induced runoff for upland (top line) and lowland (bottom line) soils at Dogwood.

The QUICKBASIC® statement IF NEWTABLE<1.3 THEN FLARVMORT = 1 produced 100% mortality for larvae stranded when no standing water occurred at Dogwood. Also, for upland strata, FLARVMORT is set equal to 1 when NEWTABLE < 1.8, because lower water levels provide flushed larvae with no access to standing water (based on a topographical map of Dogwood - see Fig. 7-1). Mortality at lowland sites is slightly lower for upland runoff-hatched larvae (Fig. 6-9); the large numbers of larvae that readily flushed from upland soil in trough studies (Ch. 4) and the extensive nature of sheetflow runoff suggest that larvae may be more likely to be flushed by runoff to nearby pools at upland sites.

Survival of larvae/pupae in the open water. Survival of larvae/pupae is estimated based on data from the literature and personal observations at the Dogwood and April study sites. Larval survival estimates are based on the method Focks et al. (1988) used to estimate larval survival in Ps. columbiae. A nominal survival of 0.60 (60% larvae survive with no predation) is decremented linearly with respect to the age of standing water due to the increased predation by growing larvivore populations. Establishment of larvivores reduces overall larval survival drastically. Focks et al. (1988a) used 0.12 for Ps. columbiae in rice; other values ranging from 0.01 to 0.07 are reported for larval survival

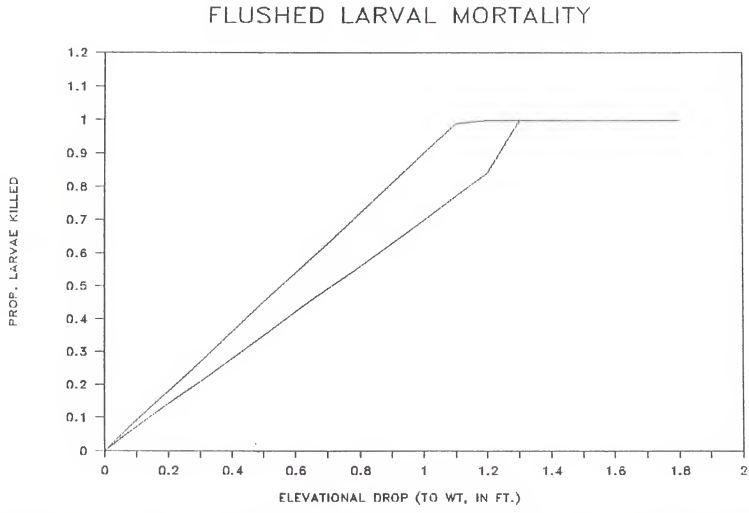



Figure 6-9. A plot of the TAENISIM estimates of the mortality of flush-hatched larvae vs elevational drop (see text) for upland (top line) and lowland (bottom line) areas at Dogwood.

in permanently-flooded rice fields (Service 1977, Mogi et al. 1984). The latter represent conditions where established populations of larvivorous fish can reduce mosquito larvae populations significantly.

The potential impact of fish is used to generate two larval survival functions in TAENISIM. In sites initially flooded only by rain, predator populations in mangrove basin forests typically include insects of the families Belostomatidae, Nepidae, Dytiscidae and Hydrometridae (Purcell 1980) and the killifish Rivulus marmoratus (Poey) and Fundulus confluentus Goode and Bean, both of which can hatch from aestivating eggs (Harrington 1959, Ritchie and Davis 1986). Initially, however, these fish have little impact on larval survival because they are too small to consume many larvae. Trapping studies suggest that populations of these fish increase more slowly than populations of live-bearers such as Gambusia affinis Girard and Poecilia latipinna LeSeur that are often introduced by tidal flooding. Thus, larvivorous fish populations at tidally-flooded mangrove basins are composed initially of larger sized individuals than are populations at rain-flooded basins; are subject to more rapid production of fry and are characterized by larger, denser fish populations that have a greater effect on the survival of mosquito larvae. Fish trap collections illustrating the larger density of fish at a tidal vs rain-flooded mangrove basin

are plotted in Fig. 6-10. Additionally, the large number of fry produced by G. affinis was observed to consume 1st instar Ae. taeniorhynchus larvae concealed in thick shoreline litter that otherwise protects larvae from predation by adult fish (Ritchie, personal observation). This is incorporated into TAENISIM by reducing minimum overall survival from 0.10 to 0.05 for tidally-flooded sites. Thus, larval survival (LARVSURVIVE) in basins only flooded by rain (Eq. 6-13) differs from LARVSURVIVE in basins flooded by the tide (Eq. 6-14).

LARVSURVIVE = 1 - (0.4 + (0.06 X FLOODTIME)) (6-13);

LARVSURVIVE = 1 - (0.5 + (0.15 X FLOODTIME)) (6-14);

where FLOODTIME = the time since inundation.

Minimal survival is set at 0.10 and 0.05 by an IF statement for rain and tide-flooded basins, respectively. Both LARVSURVIVE rates are shown in Fig. 6-11.

Larval/pupal development rate. Larval/pupal development rate is calculated from observations of broods reported by Nielsen and Haeger (1960), data presented by Nayar (1985), and personal observations. Development time is also a sine function and is estimated in TAENISIM by Eq. 6-15.

EMERGETIME = 1.35 + (0.7 X sine(2Pi X (T + 10)/52)) (6-15).

An IF statement is used to minimize EMERGETIME at values

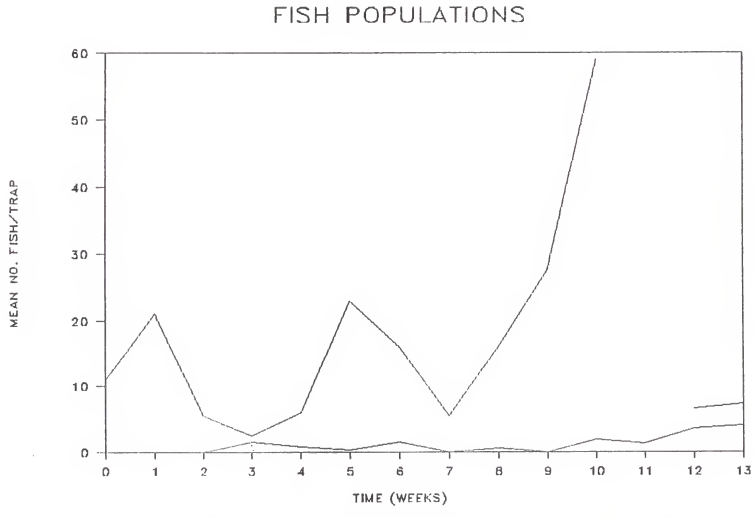



Figure 6-10. A plot of mean weekly fish collections (4 traps per week) at a tidally-flooded site (April; top line) and a rain-flooded site (Dogwood; bottom line) from late May to late August 1987.

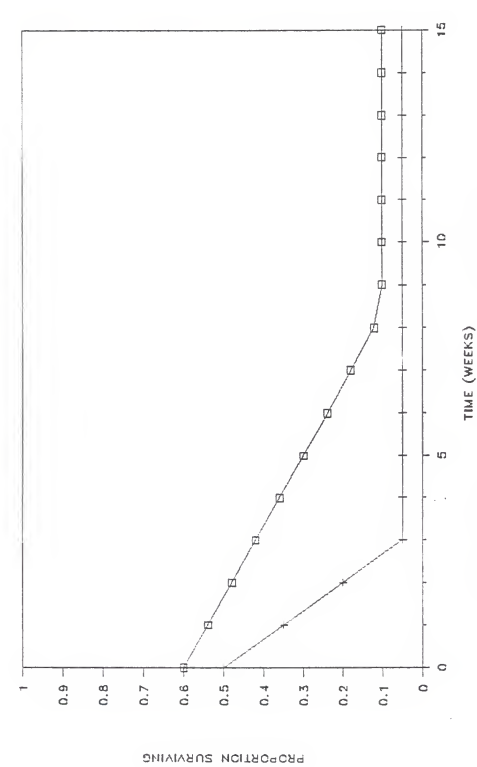



Figure 6-11. A plot of the estimates for larval survival in rain-flooded and tidally-flooded basins used in TAENISIM.

greater than or equal to 0.90 week. Values for EMERGETIME are shown in Fig. 6-12.

Adult females. Only sex ratio and survival are used for adult Ae. taeniorhynchus levels. Oviposition and associated auxiliaries are considered to control egg level inflow and are discussed under rate equations for egg levels. Although Ae. taeniorhynchus is a migratory species (Provost 1953), net migration is assumed to be zero. It is anticipated that later versions of the model will incorporate migration.

Adult sex ratio. Sex ratio is used to eliminate males from the adult levels, thus creating an adult female level. Sex ratio is assumed to be a constant of 1:1 (Nayar 1985), so the number of emerging adults is multiplied by 0.50 to determine number of emerging females.

Adult female survival. Adult female survival (ADULTSURVIVAL) is estimated using daily survival estimates that Nayar (1985) calculated from mark-release-recapture studies conducted by Provost (1953) and Bidlingmayer and Schoof (1957). Daily survival of females was found to be 0.81 at Savannah, Georgia, and 0.77 at Sanibel Island, Florida, during late August to early September; these figures equate to respective weekly survival rates of 0.23 and 0.16. Nayar (1985) suggests that survival rates may be higher in cooler seasons. Consequently, the survival rate

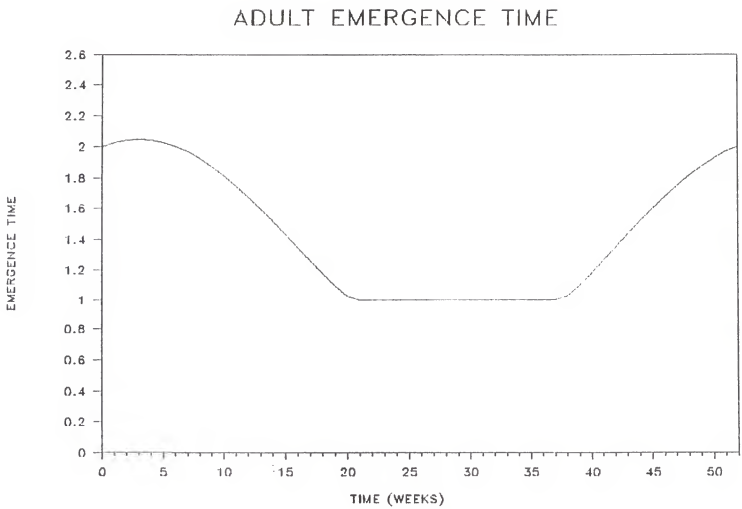



Figure 6-12. A plot of the estimates for adult emergence time (i.e., length of larval developmental period) used in TAENISIM.

function used in TAENISIM incorporates higher adult survival rates in the winter (Eq. 6-16).

(6-16) ADULTSURVIVAL = 0.35 + (0.20 X sine(2Pi X (T + 10)/52)).

Values for ADULTSURVIVAL range from 0.15 to 0.55 and are plotted in Fig. 6-13.

Sensitivity Analysis

Analysis of model sensitivity consisted of examining the relative change in a specific model population-estimate caused by fixed changes in parameters, using a standard model run. A standard model run (Fig. 6-14) was developed with the interests of mosquito surveillance and control in mind. Typically, late spring features dry weather interspersed by one or two heavy rains leading into the rainy season. This partially floods mangrove basins, hatching a moderate brood of Ae. taeniorhynchus. This inital brood produces eggs that may engender the season's largest larval population with the advent of the rainy season in June. Thus the standard run consisted of two sequential hatches timed to produce the largest hatch. The model output of interest in the sensitivity analysis was the maximum mature egg population (1463) produced by the second brood. Sensitivity analysis (Montague et al. 1982) involved doubling and halving rate parameters, then summing the percentage change in maximum egg population from the standard output. Sensi-

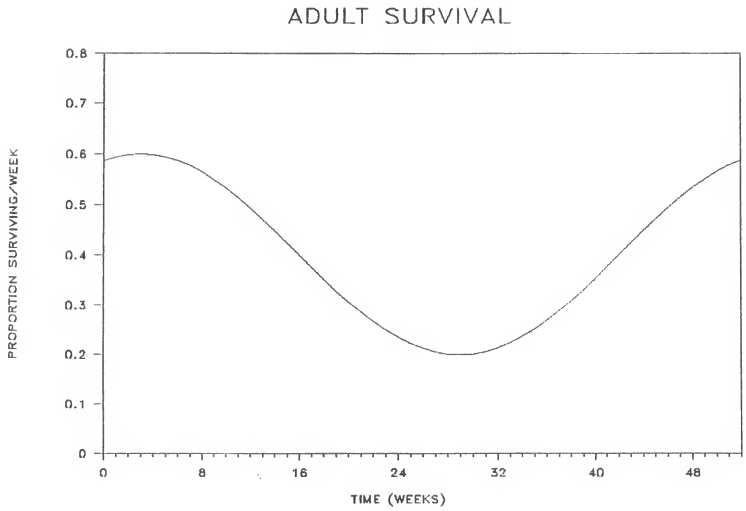



Figure 6-13. A plot of the estimates for adult survival used in TAENISIM.

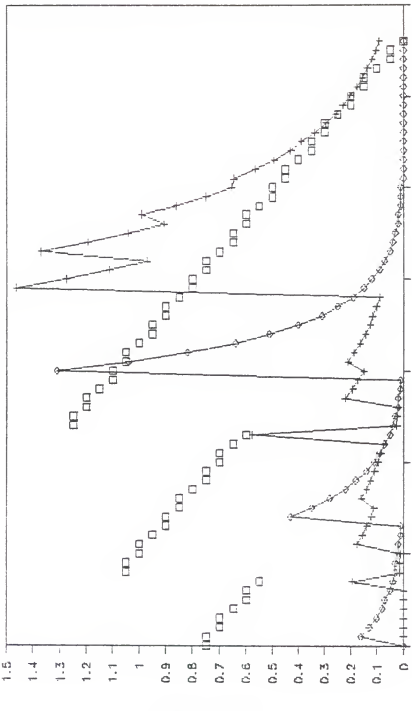



Figure 6-14. A plot of the output from the standard run used in sensitivity analysis of TAENISIM. The values represent 50X the water table (ft MSL), 10X the number of adult females and 1000X the number of eggs. The highest peak in egg number (1,463) at day 40 was the output used in the analysis.

tive parameters (total % change > 50%) were then adjusted by ± 10% and the percentage change in output summed. Parameters were then ranked by greatest total change for both procedures. The model was run on an IBM AT® personal computer with Microsoft QUICKBASIC®.

Results of the sensitivity analysis (Table6-3) provide insight into control mechanisms of Ae. taeniohrynchus population dynamics as well as into behavior of the model. Clearly the relative impact of parameters such as submerged egg mortality, larval development rate and egg maturation time are biased by the constraints of the standard run and model construction. The standard run was conducted using late spring conditions; these would maximize hatching, thereby minimizing the impact of mortality of submerged eggs. Also, the standard run featured a hatching sequence that minimized the time spent as an egg, thereby underestimating the impact of egg survival. By simply delaying the second hatch by one week, the impact of doubling the egg survival rate increased the percent change in standard run egg production from 133 to 204%. Finally, the impact of larval development time was biased because larval survival was calculated independent of developmental time.

Table 6-3. Sensitivity analysis of TAENISIM. Cumulative percentage change in standard run output (maximum egg population following second hatch, standard value = 1,463) for doubling and halving and ± 10% rate value. ND indicates procedure not done.

| | Parameter change | | | |
|---|---|---|---|---|
| | Halving, Doubling | | + 10 % | |
| Rate Parameter | Cum. change | rank | Cum. change | rank |
| I. Eggs | | | | |
| A. eggbatch | | | | |
| 1. anautogenous | 492 | 3 | 45 | 3 |
| 2. autogenous | 117 | 7 | 12 | 8 |
| B. preovip. time | 1746 | 1 | 48 | 2 |
| C. egg matur. time | 119 | 6 | 14 | 7 |
| D. mortality | | | | |
| 1. submerged eggs | 2 | 9 | ND | ND |
| 2. exposed eggs | 220 | 5 | 30 | 6 |
| E. Percent hatch | ND | ND | 32 | 5 |
| II. Larvae | | | | |
| 1. survival | 349 | 4 | 37 | 4 |
| 2. devel. time | 89 | 8 | 9 | 9 |
| III. Adult females | | | | |
| 1. survival | 604 | 2 | 76 | 1 |

The results also indicate that simulated mosquito production is fueled by rapid oviposition and adult survival. Fixed changes in preoviposition time and adult survival had the greatest impact on model output. This

suggests that Ae. taeniorhynchus could maximize reproduction by minimizing preoviposition time; the maximum number of adults deposit the maximum number of eggs. Perhaps this explains the propensity of this mosquito and many others to produce eggs autogenously (O'Meara and Edman 1975), because this would eliminate time spent sequestering and assimilating a bloodmeal, especially where hosts are rare.

Structural sensitivity analysis was conducted on the runoff hatching and larval survival parameters. In the former, the impact of no runoff hatching on maximum egg population of the second hatch was tested. Removal of this structure reduced the maximium egg population by only 3%. This probably reflects the fact that the low water levels used in the standard run resulted in little runoff hatching; eggs would be deposited primarily in lowland sites relatively free of runoff hatching. Certainly this parameter would have greater impact when the mangrove basin is fully flooded and eggs concentrated on upland soils subject to sheetflow runoff. In the analysis of the larval survival parameter, the pool was not allowed to dry before the second hatch, thereby exposing these larvae to established populations of fish not present in the standard model run. In this situation, maximum egg production was reduced by 40%. This suggests that the presence of aquatic predators may be a significant factor defining the population dynamics of Ae. taeniorhynchus.

Finally, the sensitivity analysis illustrates which parameters have the greatest potential impact on population dynamics and can serve to prioritize areas for research. This analysis suggests that precise estimates are particularly needed for stage-specific survival rates (egg, larva-pupa, adult). But more importantly, greater emphasis should be placed on the stage; reliable estimates of egg batch size, proportion autogenous and oviposition frequency are necessary for realistic model prediction.

## Discussion

Construction of TAENISIM, while laborious and Sisyphean, did offer reward. Several new techniques, developed in the course of estimating rate parameters, should enhance mosquito ecology research. Feedback dynamics served as a method to describe, synthesize and test hypotheses concerning the complex population dynamics of Ae. taeniorhynchus. Sensitivity analysis of the model pinpointed life history events that have the greatest potential impact upon population dynamics. This served to highlight areas necessitating further research.

Laboratory and field studies of Ae. taeniorhynchus eggs produced novel techniques and information of use in mosquito ecology. Silicone caulk was found to be useful in exposing known numbers of eggs to predators in the field and lab. Eggs can be placed in or out of water and on a variety of natural substrates. Most importantly, eggs are

strongly adhered and thus provide an exact count of the number of eggs lost by predation and senescence. This technique was used to show that the survival rates of exposed eggs vary seasonally and appear to be greatly affected by predation. Also, studies indicated that submerged immature eggs will mature and will have higher survival than that of exposed eggs.

Much work must be completed before TAENISIM can be accepted as a valid model. The model must be run using real or estimated hydrologic data from Dogwood. The model output must appear realistic and comparable to field estimates. The hydrology model used in conjunction with TAENISIM will be presented in Ch. 7, and a complete validation of TAENISIM will be presented in Ch. 8.

CHAPTER 7
A HYDROLOGICAL CHARACTERIZATION AND SIMULATED WATER LEVEL FOR A FLORIDA MANGROVE BASIN FOREST

## Introduction

The ultimate goal of this project was the development of a simulation model to predict hypothetical responses of area relationship and water level (i.e. depth of free-standing water) for the Dogwood basin. This chapter decribes how topographical and hydrological features of Dogwood were used to determine the Dogwood stage-area relationship (cumulative area flooded for a specific water level stage) and were incorporated in an electronic spreadsheet model (Hancock and Heaney 1987, Rowan et al. 1988) to estimate Dogwood water levels. Additionally, the validity of water table well data in providing water level estimates at Dogwood was examined. It was hoped that the hydrology model could overcome the limitations of the well data.

Several topographical and hydrological parameters were measured and estimated at Dogwood. Characteristics of interest were cumulative area for a specific elevation, height of basin overflow, height of flooding tide, evapotranspiration-infiltration (ETI) and relationship of rainfall and tide to changes in water level (note that the term

water level is used since the objective of the model is to simulate the depth of free-standing water in the basin). Field estimates of these parameters were used to develop a spreadsheet simulation model to predict Dogwood water levels. Well records and measurements of water level were used to validate the model. Finally, the model was used to generate data used in TAENISIM to predict Ae. taeniorhynchus populations for TAENISIM validation.

## Materials and Methods

### Hydrological Characteristics of Dogwood

The topographical and hydrological characteristics of the Dogwood basin necessary for construction of TAENISIM and HYDROMOD were estimated. Basic topographical data included maximum and minimum basin elevations and surface area of the basin. A computer-generated topographic map of the site was developed, and was used to determine elevational stage vs area and stage vs cumulative area relationships. Hydrological characterization include estimation of daily water loss due to ETI and quantification of the relationship of rain and tide to changes in water level.

Elevations at Dogwood were measured and used to produce a topographic map and to estimate stage-area relationships. Due to the logistics of measuring elevations over a large area (the size of the basin was 1.75 acres (0.71 ha)), elevations were only measured within a

120 X 180 ft grid (36.6 X 54.9 m) that encompassed the full range of elevations at Dogwood. Data were extrapolated from the grid to the entire basin with the aid of an aerial photograph and personal observations of inundations.

Elevations within the grid were estimated from elevations obtained with a level and transit during Novembver, 1984. Elevations were measured at the corner of every grid square (total of 48 squares) and at perceived high and low spots within the grid; a total of 109 measurements was made (see Appendix F). A topographical map of the grid was generated by SURFER® (Golden Software, Inc. 1987), the elevation of estimated grid points was calculated using the Kriging gridding algorithm and contour were lines smoothed by cubic splining; default settings were used for both procedures. This program produced an ASCII file of the 640 estimated elevation points that was input into LOTUS 123® and a frequency distribution of elevation at 0.20 ft intervals was obtained and used to estimate the stage-area relationship for the entire basin.

Specifically, four topographical zones were identified and three were used to estimate elevations from the grid. First, the lowest areas (1.2 - 1.6 ft above mean sea level (MSL)) were located in a shallow, mud-bottomed depression. Since this zone was entirely the grid, no extrapolation to areas outside the grid was necessary. Second, the middle elevations (1.6 - 2.8 ft MSL) comprised most of the

basin and consisted of scattered red mangrove hummocks and interconnecting low spots. Third, a gently sloping region encircled the lower two regions forming a uniformly wide band of highest elevations (2.8 - 3.4 ft MSL) around most of the basin. Fourth, a steep bank drops off into the basin on the west side of the grid (see p. 10; Fig. 2-1). Data from this area were not incorporated into the basin stage-area relationship because this area was never flooded.

Thus, calculation of stage-area involved three steps. First, low elevations were simply tallied from the grid. Second, highest elevations were assumed to encompass a band of constant width (as measured within the grid) around most of the basin. The area of the consecutive bands for elevations at 0.2 ft intervals was calculated from 2.8 to 3.4 ft (height at which mangrove vegetation is replaced by hammock vegetation). One small region of the periphery featured a steep bank and the area was appropriately calculated using a much narrower band width. Finally, the remaining basin area contains the mid elevation zone. The area of this zone was estimated by subtracting the low and high zone areas from the total basin area. The distribution of stage specific area within this region, assumed to be identical to the distribution of middle elevations within the grid, was directly extrapolated from the grid data.

A cumulative stage-area model was developed in LOTUS 123® to predict areal flooding for a given water level. Curve fitting of the model to field data was based on the maximal value of $r^2$ for a regression of model output against the Surfer®-derived elevations. The model was updated using a new low elevation because measurements taken in 1987 indicated that the lowest elevation had increased from 1.2 to 1.5 ft MSL.

## Hydrological Model of Dogwood

### Statistical analysis of well data

Well data were collected initially to provide a record of the water level at Dogwood and April during the course of the project. Unfortunately, the wells frequently produced readings conflicting with observations of basin water levels. The April well invariably failed to record basin flooding by tidal inundations accurately; the Dogwood well was so strongly influenced by tide that the impact of rainfall was often masked. Apparently, both wells, located uphill from the basin, measured a water table that was distinct from the basin water level, particularly during tidal flooding and when water levels were low.

Therefore, actual water level readings were compared with well readings in an attempt to calibrate well data. Staff gauges were installed at the lowest spot for each basin at the resulting water level and paired readings of water

level and well water table recorded. These data were regressed and, if a significant regression was obtained, were used to calibrate water level from daily high well readings. Unfortunately, results for the April basin were so poor ($r^2 = 0.33$) that well data could not be used to estimate water levels accurately. Hence, further water level analysis will only involve the Dogwood basin.

Spreadsheet simulation model of Dogwood water levels

A simulation model of the Dogwood basin water level was constructed to overcome the limitations of the well data. An electronic spreadsheet, similar to the model developed by Rowan et al. (1988) to predict water levels at an east Florida impoundment, was used. Field estimates of changes in the water level due to ETI, rain, tide and basin topography were incorporated into the model. The model was validated by comparison with water levels measured by a staff gauge and by observations of critical water levels (drydown and spillover).

Estimation of hydrological parameters at Dogwood. The staff gauge readings used in well calibration were used to estimate ETI and the impact of rain on water level. Water level readings were taken from 24 May - 26 August 1987; 75 readings were taken. Rain measurements were obtained daily from the Barfield Dr. Fire Station located ca. 100 m from Dogwood. The mean change in water level on days with no

rain provided an estimate of ETI. The relationship of 24-hr rainfall to 24-hr change in water level was examined by regressing daily rain against change in water level for days featuring rain.

The impact of tide on Dogwood water levels was estimated by well and tide records and by observations of flooding tides. Tide records were obtained from a Stevens® recording tide gauge located within 1 mile of Dogwood in Barfield Bay. The minimal high tide necessary to flood Dogwood was determined by observing the water level height for inflow and outflow at Dogwood. Well readings indicate that the Dogwood water table oscillates relative to the tide, while no oscillation was observed in free-standing water. This phenomenon could support higher water tables during dry periods. The relationship between water table and tide was estimated by regressing daily high tide and well readings for a period when the basin was dry with no major (> 0.50 cm) 24-hr rain.

Development of spreadsheet model (HYDROMOD). The hydrological estimates were used to generate the spreadsheet simulation model. In general, the model estimates current water level by adding the calculated change in water level to the previous day's water level. Change in water level is composed of the following (variable name followed by typical change value): ETI (-), RAIN (+), TIDE (+,+-) and

SPILLOVER (-). ETI results in a relatively consistent water loss. RAIN acts as a pulse increase in WATER LEVEL proportional to rainfall. TIDE acts in two ways. First, it increases WATER LEVEL dramatically by flooding the basin; second, it supports an oscillating WATER LEVEL when the basin is dry. SPILLOVER tends to rapidly decrease WATER LEVEL when extremely high tides and heavy rains overload the storage capacity of the basin.

These variables are placed in columns atop the spreadsheet and used to calculate change in WATER LEVEL, the final model output. Additional inputs (i.e. spreadsheet columns) are 24-hr rainfall, 24-hr high tide, and calendar date by week. Additionally, certain columns are used to correct model variables, for example ETI is corrected for time of year since evapotranspiration values change with the season (Smajstrla et al. 1984). The model time step is one day, with each row corresponding to a daily recalculation of the Dogwood water level. A line listing of the HYDROMOD model is available in the Appendix G.

HYDROMOD calibration. Model calibration involved comparing model output for a January - August 1987 run with water levels recorded from May - August. The 1 January water level at Dogwood was easily initialized since an extremely high tide had flooded the basin; the level of the tide was used as the initial value of basin water level. A graph of

the predicted and actual water level and the mean absolute difference between the two water levels was used to guide calibration of the model. Model output was altered by changing values of variables used in the model. Model performance was examined by comparing the mean and standard deviation of predicted and actual Dogwood water levels.

## Results

### Hydrological Characterization of Dogwood

#### Topographical description

A topographical map (Fig. 7-1; data points provided in Fig. 7-2) and surface contour plot (p. 10; Fig. 2-1) of the Dogwood grid indicate the complex topography of the basin. Four distinct topographical regions are visible, especially in the surface contour plot Looking at the surface contour plot, one can distinguish a steep bank that drops off into a basin characterized by an undulating landscape formed by the prop roots of red mangrove trees. The low region in the lower left hand corner of Fig. 7-2 represents the lowest area of the basin (elevation 1.2 ft MSL). The mid elevation areas (1.6 - 2.6 ft MSL) give way to a uniformly-sloping area on the right hand side of the plot; this upland region (2.6 - 3.4 ft MSL) represents a transition from red mangrove to mixed red and black mangrove to, ultimately, tropical hammock vegetation.

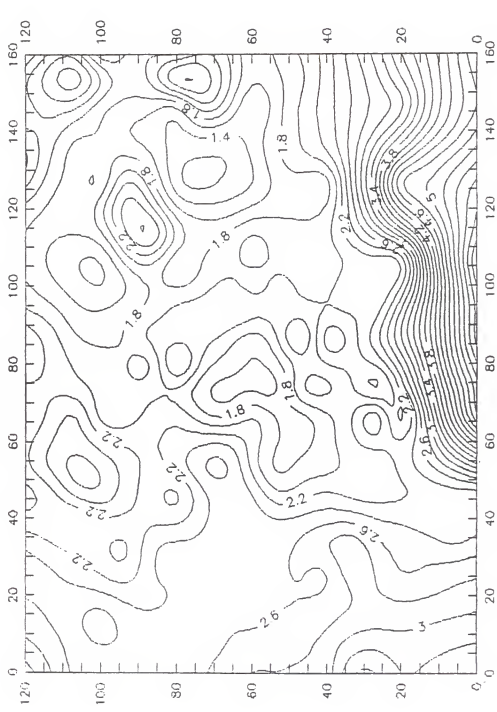



Figure 7-1. A plot of a topographic map of the Dogwood grid (generated by Surfer®). Axes are in ft and elevations are in ft above mean sea level. Contours are in 0.2 ft increments. The left edge of the plot faces north.

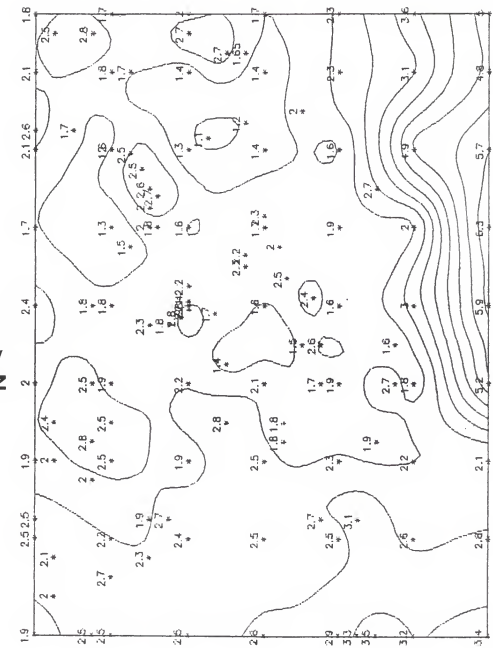



Figure 7-2. A plot showing the elevations (in ft above mean sea level) used to produce the topographic map (Fig. 7-1) and surface contour plot (Fig. 2-1) of the Dogwood grid. Contour lines (0.5 ft increments) are provided for reference.

The red mangrove hummocks appear to have a significant impact on stage-area relationships for Dogwood. A graph of stage vs cumulative area (Fig. 7-3) indicates that surface area increases at highest rates at elevations 2.0 - 2.4 ft MSL, the region dominated by red mangrove hummocks. Clearly this region offers considerable area for mosquito oviposition and for ponding critical to mosquito production (Rowan et al. 1988). Stage-area then increases linearly as hummock peak elevations are reached (elev. 2.4 - 2.8 ft MSL) and the uniformly sloping upland areas are flooded. Observations suggest that undulating and gently-sloping contours are characteristic of impounded red and black mangrove forests, respectively.

A function was developed from the stage-area (refers relationship to predict cumulative area for a given stage. The function (Eq. 7-3), reflecting the diversity of the stage-area relationship, consisted of two components. For elevations below 2.6 ft MSL, cumulative area increased exponentially with respect to stage. Equation 7-1 was used to estimate cumulative area for elevations < 2.6 ft MSL;

$$A(H) = 1.6/(1+(50e^{-4(H-1.36)})) \qquad (7\text{-}1);$$

where A(H) = area flooded (in acres) for the Hth stage and

H = basin stage, in feet MSL.

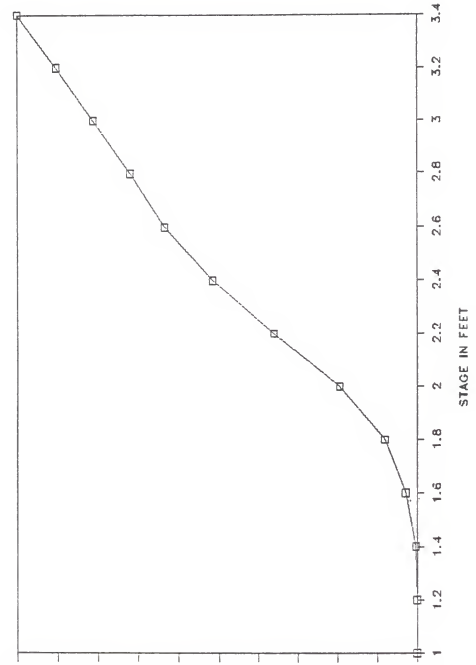



Figure 7-3. A plot of the stage-cumulative area relationship, based on Surfer® elevation estimates, for the Dogwood basin.

When output from Eq. 7-1 was regressed against elevations estimated by Surfer$^{R}$, an $r^2$ of 0.9992 was obtained. A linear model (Eq. 7-2) was used to estimate cumulative area for elevation of 2.6 ft MSL:

$$A(H) = -0.98 + 0.8(H) \qquad (7\text{-}2).$$

This equation produced an $r^2$ of 0.9996 when regressed against the Surfer$^{R}$-estimated elevations. In the spreadsheet, these functions were combined to produce the following conditional function (Eq. 7-3) based on stage:

$$A(H) = @IF(H<2.6,1.6/(1+(50e^{-4(H-1.36)})),$$
$$@IF(H>=2.6,-0.98 + 0.8(H)) \qquad (7\text{-}3).$$

This function (MODEL 1 in Fig. 7-4) had an $r^2$ of 0.999. Use of the exponential function (Eq. 7-1) alone produced an $r^2$ of 0.989 when regressed against the estimated elevations. This suggests that an exponential function may well be characteristic of stage-cumulative area relationships in similar mangrove basins.

During calibration of the staff gauge in July 1987, it was found that the elevation of the basin's low spot had increased from 1.2 to 1.5 ft above MSL. Thus, the exponential model was altered to account for this increase. The new model (MODEL 2, Eq. 7-4),

$$A(H) = 1.75/(1+e^{-5 \times (H-2.45)}) \qquad (7\text{-}4)$$

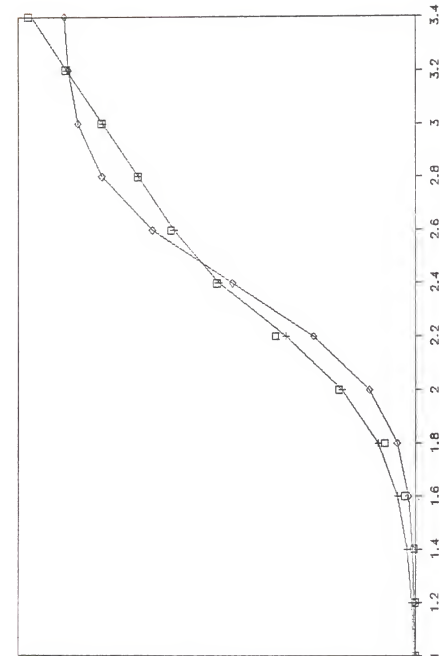



Figure 7-4. A plot of the stage-area relationships for the Dogwood basin. Connected symbols are estimates (MODEL 1 and MODEL 2) and squares are values estimated using SurferR.

produces a steeper response of cumulative area to slope, reflecting the decreased elevational range of the area. A plot of the models and Surfer® data points is shown in Fig. 7-4.

The development of MODEL 2 demonstrates the application of a simple exponential function to describe stage-area relationships. The general form of the exponential is represented by hydrological parameters as follows:

$$A(H) = \text{BASIN AREA}/(1+e^{(\text{SLOPE FACTOR} \times \text{STAGE} - \text{STAGE MEDIAN})})$$

where SLOPE FACTOR is usually a negative value for which smaller values increase slope; STAGE is the elevation for which cumulative area is desired; STAGE MEDIAN is the elevation at which the basin is 50% full. Obviously this model represents a gross simplification of reality; it assumes that cumulative area is symmetrical about the median stage when, in reality, cumulative area is probably skewed to the right of the median stage. However, it does feature a constant cumulative area maximum characteristic of most basins and it is simple to construct.

Statistical analysis of well data

Well data were originally collected to provide a record of Dogwood water levels. This section describes the well's performance compared to staff gauge records and

personal observations. The regressions of daily high well reading and daily high well X well$^2$ against staff gauge water levels were significant ($P < 0.01$) but the resulting $r^2$ (0.84, and 0.86, respectively) indicate a less than perfect fit (Fig. 7-5). The variability in the data reflects the interaction of tide and water table at Dogwood. Values of the Y intercept (1.85 and 2.04 for the linear and quadratic models, respectively) were greater than the elevation of the bottom of the basin (1.5 feet) indicating that the well water table reading diverged increasingly from staff gauge readings as the basin water level decreased. This is probably due to the upward sloping of the water table as the well's height relative to the basin water level increases.

As mentioned earlier, the subterranean water table was subject to variation due to tide. Figure 7-6 shows the interaction between tide and daily high well readings at Dogwood during late spring 1986, a period with no significant (i.e. > 0.5 cm/24 hr) rainfall. Clearly, tide affects both the peaks and the mean water table values. The water table responds similarly to daily tidal rhythms.

From these analyses, it was apparent that the well data provided an inaccurate record of the Dogwood water level. This necessitated development of a spreadsheet hydrologic model to calibrate recorded well data and predict Dogwood water levels from hypothetical rain and tide.

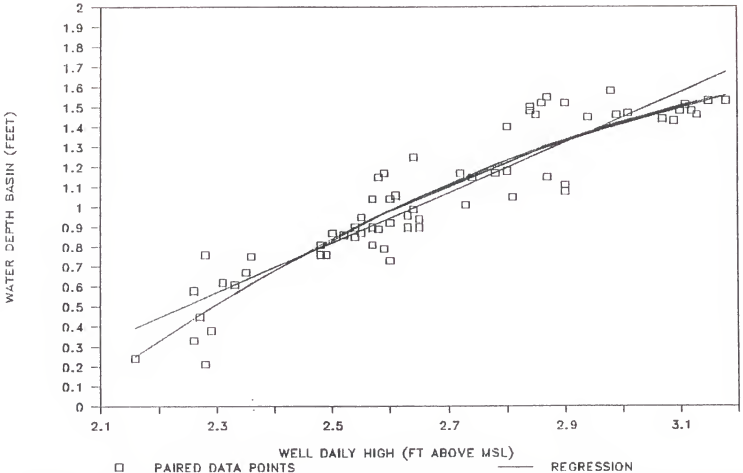



Figure 7-5. A plot of paired water table well (highest daily reading) and staff gauge readings at Dogwood. Lines are regression estimates of water depth of the basin from well data (Y = -2.31 + 1.25X; Y = -7.35 + 5.03X + 0.70Xsquared).

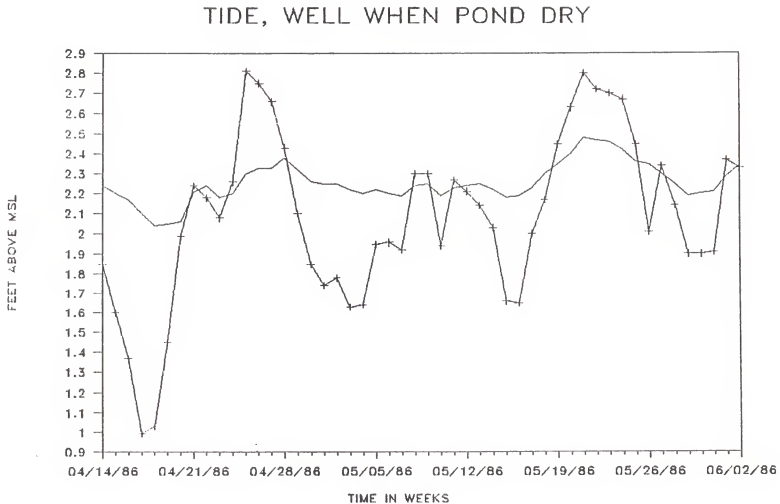



Figure 7-6. A plot of Dogwood water table well daily high (line) and Barfield Bay daily high tide (connected symbol). Observations are from a period when the basin was dry and when no rain exceeding 0.5 cm fell over a 24-hr period.

Simulation Model of Dogwood Hydrology (HYDROMOD)

Estimation of hydrological parameters

Summer values of ETI and the relationship of rain and tide to change in the Dogwood water level were estimated for use in a spreadsheet simulation model (HYDROMOD) of the Dogwood water level. The parameters were estimated from 75 staff gauge readings obtained from late May - late August, 1987.

Water level change due to ETI was estimated by changes in water level on days with no rain. The mean $\pm$ SD daily change in water level for rainless days was -0.036 $\pm$ 0.024 ft. The regression of daily water level change on rainless days vs water depth was not significant, suggesting that ETI is independent of free-standing water depth.

The relationship of rainfall to change in water level was also estimated from the summer staff gauge data. Before analysis, data pairs featuring (1) no measurable rain and (2) a water level at or above basin spillover elevation (i.e. 1.55 ft) were excluded from the analysis. Remaining paired rain and water level change observations were regressed. Significant regressions were obtained for rain and rain X rain$^2$ vs water level change ($P< 0.01$, $r^2$ = 0.749 and 0.754, respectively). For the linear model, change in water level = -0.08 + (0.20 X rain (in inches)); the results suggest that light rains (< 0.08 in) do not increase water level. For the quadratic model, change in

water level = -0.10 + (0.24 X rain) + (-0.02 X rain$^2$); this model similarly indicates that small amounts of rain do not increase water level. A plot of the data and the linear model is shown in Fig. 7-7.

The interaction of tide and water levels at Dogwood is dynamic; tides serve to flood the basin and to support the water table during dry periods. Observations of flooding tides at Dogwood and daily high tide suggest that tides in excess of 3.2 ft MSL cause complete inundation of the basin. While high tides of 3.1 ft MSL can overtop the Dogwood berm, they do not result in complete inundation. Well readings during dry periods (Fig. 7-6) clearly show how tides can support and even increase the subterranean water table during dry periods. Staff measurements indicate that free standing water does not fluctuate with the tide. However, the impact of the tide on the water table must be accounted for in HYDROMOD since high late-spring tides may maintain a high water table despite drought conditions, enhancing the likelihood of flooding and mosquito hatching with initial rainy season storms. Figure 7-6 suggests that a response lag between high tide and water table rise occurs. A regression of daily high tide vs daily high well readings confirms this. The respective $r^2$ for high tides 3, 2, 1 and 0 days before the well reading was 0.32, 0.54, 0.72 and 0.66. This indicates

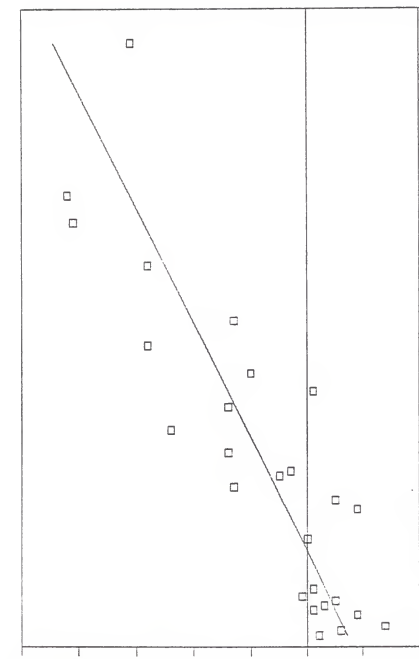



Figure 7-7. A plot of the relationship between 24-hr rainfall and the change in water level (i.e. staff gauge reading) at Dogwood. The regression line, Y = −0.08 + 0.20X, is shown.

that a 1-day time lag in daily high tide best accounts for variation in "dry basin" well readings.

Construction of Spreadsheet Hydrological Model (HYDROMOD)

Estimates of the hydrological parameters discussed above were incorporated into a spreadsheet format that calculated potential changes in Dogwood water level. As expected, each parameter was altered, within limits of perceived realistic shortcomings of the field estimates, in order to maximize the fit of model output to field-collected water levels. The following section discusses the rationale for and development of the updated model components.

Explanation of model formulas involves several different variable names and symbols; familiarity with LOTUS 123® is advisable. Formulas are provided in standard mathematical and LOTUS 123® format. Variable names are capitalized (Table 7-1) and followed by subscripts, (#) or (#-1), that respectively refer to column row (i.e. day) and previous column row (i.e. previous day) in the spreadsheet. Conditional statements are used and are in the format @IF(THIS CONDITION IS TRUE, THEN DO THIS, ELSE DO THIS). Note that the conditional alternatives are ordered from true to false and are separated by a comma. LOTUS 123® cell formulas are provided.

Table 7-1. Variables and variable names used in HYDROMOD

| Variable name | Definition |
|---|---|
| WEEK | Current date in weeks starting on 1 January |
| TIDE | Daily high tide |
| ETI | Evapotranspiration-infiltration in ft |
| PPT | 24 hour rain (in) |
| CETI | Corrected ETI - caps summer rates at 0.035 ft |
| CHG | Change in water level due to rain, CETI |
| CCHG | Corrected CHG - for basin spillover |
| MAXELEV | Maximum water level before basin spillover |
| WMOD | Wet model estimated water level (used when basin flooded) |
| DMOD | Dry model estimated water level (used when basin dry) |
| FMOD | Final model estimate of water level |

The model derives final water level output from two concurrent models. DMOD (dry model) describes changes in water table, as influenced by tide, for a dry basin; WMOD (wet model) describes changes in water level for a flooded or dry basin without incorporating tidal influence on a dry basin. Final model (FMOD) chooses the output that has the highest water level value.

Daily ETI rates are presumed proportional to temperature and are modeled as a modified sine function reflecting seasonal temperature changes (Eq. 7-5);

$$ETI(\pm) = 0.04+0.02 \times \sin(2Pi \times ((W-15)/52) \quad (7\text{-}5);$$

where W = the week of the year.

In the spreadsheet, the ETI cell formula (Eq. 7-5) is

ETI(#) = 0.04+0.02*@SIN(6.283*((WEEK(#)-15)/52)) (7-6).

The ETI ranges from 0.02 ft per day in late January to 0.06 ft per day in July. The field estimate of 0.035 ft per day was used to correct the data. Corrected ETI (CETI) was calculated in the adjacent column to values not exceeding 0.035 ft per day by the following statement (Eq. 7-7):

CETI(#) = @IF(ETI(#)<0.035,ETI(#),0.035) (7-7).

Figure 7-8 shows the seasonal trend in ETI. The winter low represents an ETI 57% of the maximum value, a change similar to potential ET rates for south Florida (Smajstrla et al. 1984).

The initial calculation of change in water level involved change due to rain and CETI. First, if rain had occurred, then change in water level due to rain and CETI was calculated; if no rain had occurred, change was limited to CETI. Calculation of the change in water level due to rain involved the following functions: first, 24 hr rainfall less than 0.20 in was discounted by the model by subtracting 0.20 from input rain (PPT(#)). This value was then sequentially multiplied by the following two functions:

(PPT(T)-0.2) X 0.3 and (7-8);

$(PPT(T)-0.2)^2$ 0.08 (7-9);

where PPT is 24 hr rainfall.

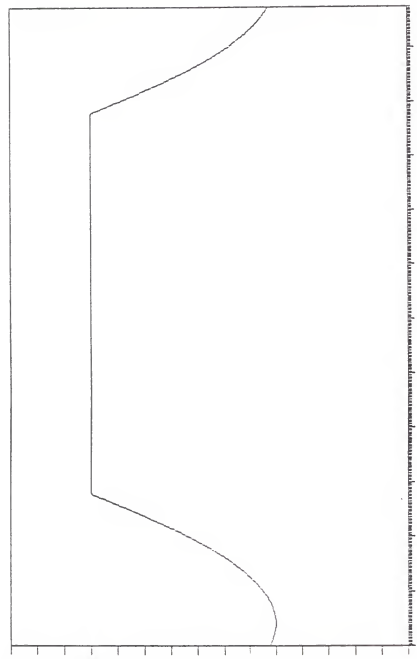



Figure 7-8. A plot showing the values of corrected evapotranspiration-infiltration (CETI) used in HYDROMOD.

The output from the two functions was then summed. These functions had the impact of (1) linearly increasing rain while (2) increasing the relative contribution of heavier rains. In HYDROMOD the water level change due to rain is calculated as follows:

```
@IF(PPT(#)>0,
(((PPT(#)-0.20)*0.30)+(PPT(#)-0.20)^2*0.08),-CETI)
```

(7-10).

The relationship of water level change to 24-hr rain is shown in Fig. 7-9.

Loss due to corrected CETI alone is also modified if the basin is dry. It was speculated that water table loss would decrease as watertable depth below the surface increased. Once open water is removed, evaporation is reduced and soil water becomes more tightly bound as the soil dries and is estimated by the following equation:

$$\text{CETI LOSS} = \text{CETI X } (1 + (H(T-1))^{4}) \qquad (7\text{-}11).$$

The spreadsheet function simulating this hypothetical relationship is as follows:

```
@IF(WMOD(#-1)>0,-CETI(#),(-CETI(#)*((1+WMOD(#-1))^4))).
```

(7-12).

This states that if the previous water level (using wet model) is greater than 0 (i.e. basin not dry), then change

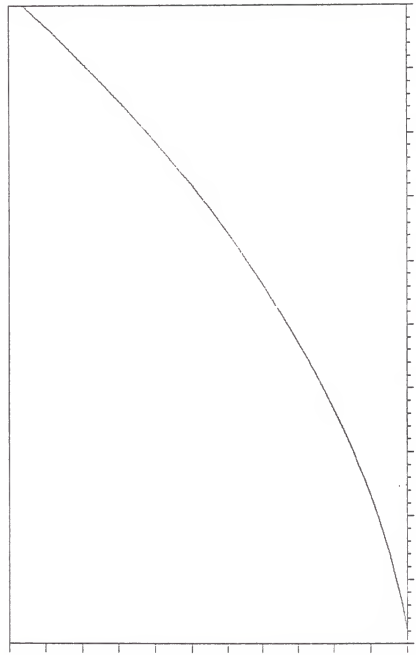



Figure 7-9. A plot depicting the relationship between 24-hr rainfall and water level change used to estimate water level changes in HYDROMOD.

in water level = -CETI; otherwise if basin is dry, then change in water level = -CETI X ((1 + previous water level)$^4$. The latter statement, since the value of the water level for a dry basin is a negative number, decreases swiftly ETI loss as the water table drops. For example, if the previous water level were -0.05, the loss due to CETI would be CETI X (1 + -0.05)$^4$ or CETI X .81; if the previous water level were -0.20, loss due to CETI would be CETI X (1 + -0.2)$^4$ or CETI X 0.41. This function serves to limit extreme falls in water tables for a dry basin.

The next spreadsheet column also corrects a potential change in water level that would result in unrealistic water levels. Clearly, as shown in Fig. 7-9, heavy rains result in an unrealistic contribution to water level change; very heavy rains need to be corrected for spillover from a inundated basin. This conditional component is based on the following rationale: if previous water level < spillover and if calculated change in water level + previous water level are greater than the maximum elevation (i.e. spillover elevation) of the basin, then the water will exit the basin and the water level will stabilize. In HYDROMOD this statement is written as follows:

```
@IF(WMOD(#-1)<MAXELEV,
@IF(CHG(#)+WMOD(#-1)<MAXELEV,CHG(#),
(MAXELEV-WMOD(#-1))+(CHG(#)-(MAXELEV-WMOD(#-1)))*0.1),
CHG(#)*0.1)                                          (7-13).
```

An example illustrates the rationale of this function. Assume the previous water level was 1.0 ft for a basin that tops over at 2.0 ft of standing water. A very heavy rain contributes an estimated 3 ft of water to the previous water level; this would result in a calculated water level of 4 ft without consideration of spillover. The first section of the function (MAXELEV-WMOD(#-1)), excluding the first conditional alternative (establishes that the rain will produce spillover), simply represents the portion of calculated water level change that would fill the basin up to the overspill elevation. In our example, this calculates to 2.0 - 1.0 = 1.0 ft. Once filled, the water spills over the basin's berm and the rate of increase in water level must decline. The equation ((CHG(#)-(MAXELEV-WMOD(#-1))) X 0.10) calculates 1) the portion of potential water level change beyond that which filled the basin to the spillover elevation and 2) multiplies this amount by 0.10, resulting in a damped change in water level beyond the spillover elevation. In our example, 3.0 - (2.0 - 1.0) X 0.10 = 2.0 X 0.10 = 0.20 ft of water is added to the already full basin. Thus, adding the two components, the corrected contribution of the rain to the previous water level is 1.0 + 0.20 = 1.2 ft. The impact of this function on the 7.25 in rain event of 27 June 1985 is shown in Fig. 7-10. If the rain had fallen on a basin at or above spillover elevation (i.e. first IF statement), all of the

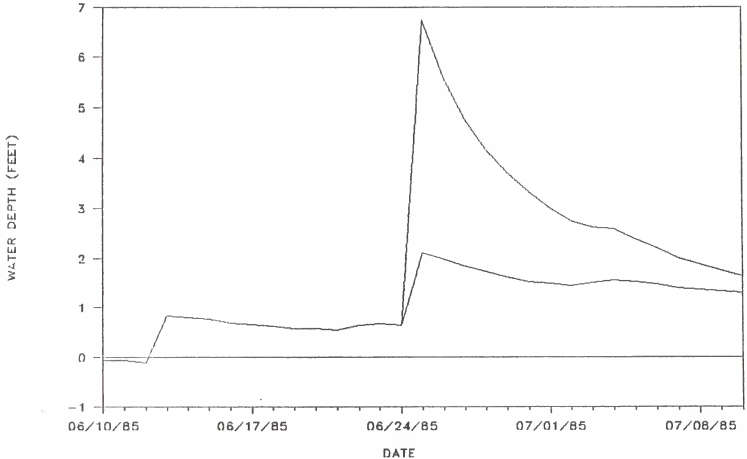



Figure 7-10. A plot showing HYDROMOD-estimated water levels that were calculated both using (bottom line) and not using (top line) the rainfall spillover function during spillover conditions (see text).

water level change would be damped by spillover; CCHG(#) = CHG(#) X 0.10.

Basin spillover may continue to act long after an extreme flooding event. Therefore, change in water level was further modified to create a rapid decline in water levels when a basin is filled beyond the spillover elevation, approximated by the following equation:

$$WL(T) = WL(T-1) + CHGWL(T) + (-0.025 \times (WL(T-1))^2) \quad (7\text{-}14);$$

where WL(T) is water level at the Tth day and
CHGWL(T) is the estimated change in water level for the Tth day.

The statement is further modified by the presence of a flooding tide that would set water level to the height of the tide. In HYDROMOD, rapid drainage of an overflowing basin is written as follows:

```
@IF(TIDE(#)-1.5<MAXELEV,
@IF(FMOD(#-1)+CCHG(#)<MAXELEV,FMOD(#-1)+CCHG(#),
FMOD(#-1)+(CCHG(#)+(-0.025*(FMOD(#-1)^2)))),
@IF(TIDE-1.5>FMOD(#-1),TIDE-1.5,FMOD(#-1))      (7-15).
```

The first conditional alternative (IF TIDE-1.5 < MAXELEV,...) sets the water level to the level of a flooding tide (i.e TIDE-1.5 >= 3.2), subtracting 1.5 from

tide elevation to correct for the height of basin bottom in ft MSL. The second conditional alternative (IF WMOD(#-1)+CCHG(#)<MAXELEV,WMOD(#-1)+CCHG(#)) states that if corrected change + previous water level < maximum basin elevation without spillover, then add corrected change to previous water level. The final conditional alternative calculates water level decrease for an over-spilling basin. Note that the rate of decline increases with the square of the water level; the role of this function in rapidly forcing water levels to capacity levels is illustrated in Fig. 7-11. The last @IF statement (@IF(TIDE-1.5>FMOD(#-1),...) sets the water level at the height of a flooding tide if that tide is higher than the previous water level. This function represents the final calculation for water level in the wet model version (WMOD).

Calculation of water level using the dry model (DMOD) is much simpler. DMOD acts a dynamic function only when the basin is dry. At this time, tidal forces support a water table that "floats" with the tide. As reported earlier, previous day's high tide was found to have the highest correlation to Dogwood daily high well readings. The regression model for this relationship was incorporated into DMOD. In the spreadsheet, DMOD calculated water level (actual level, not change in level) as follows:

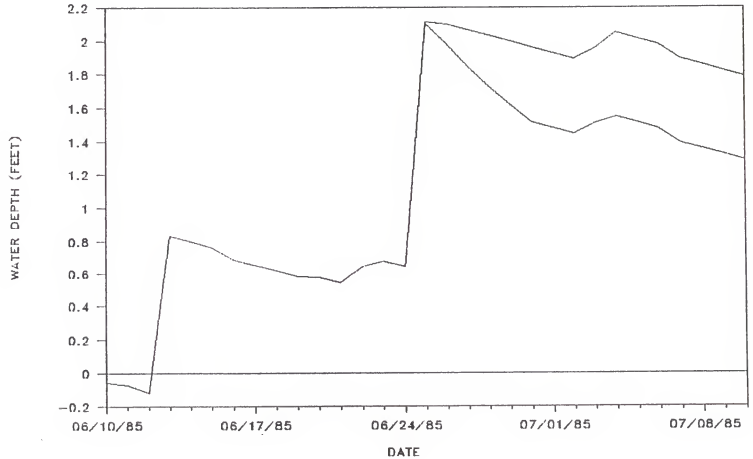



Figure 7-11. A plot depicting HYDROMOD-estimated water levels calculated both using (bottom line) and not using (top line) the basin overspill function (see text).

@IF(WMOD(#)<0,(-0.66+(0.2*TIDE(#-1)))+CCHG(#),-5)

(7-16).

Figure 7-12 shows the impact of tide and DMOD on estimated water levels for a dry basin. This equation states that if wet model water level is less than 0, then calculate dry model water level using tide regression model + corrected change; otherwise set dry model water level to -5. The value of corrected change is added to the tide model to account for the potential addition of water due to rain to a water table already supported by the tide. The latter conditional alternative sets dry model water level to a low level so that, as explained in the next paragraph, the final model will always choose the appropriate water level estimate.

The final HYDROMOD calculation (FMOD) represents the choice between WMOD and DMOD estimates of water level, i.e. the best estimate of Dogwood water level. In HYDROMOD, FMOD(#) = @IF(WMOD(#)>DMOD(#),WMOD(#),DMOD(#)). In general, the model with the highest water level estimate is the appropriate choice. Thus, setting DMOD = -5 when the basin is flooded guarantees that a WMOD water level estimate will be selected for a flooded basin.

Hydromod Calibration and Simulation Runs

HYDROMOD provided valid estimates of water levels at Dogwood for the 1987 run. Figure 7-13 shows Dogwood well

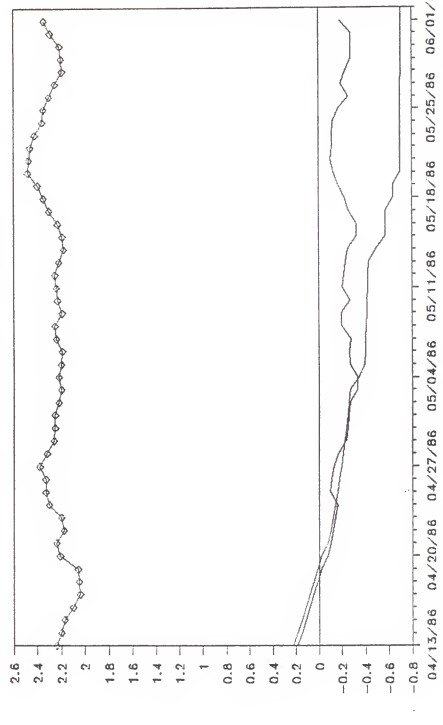



Figure 7-12. This figure shows HYDROMOD-estimated water levels calculated both using (top solid line) and not using (bottom solid line) the tide-water level function (DMOD). Daily high tide is also provided (connected symbols).

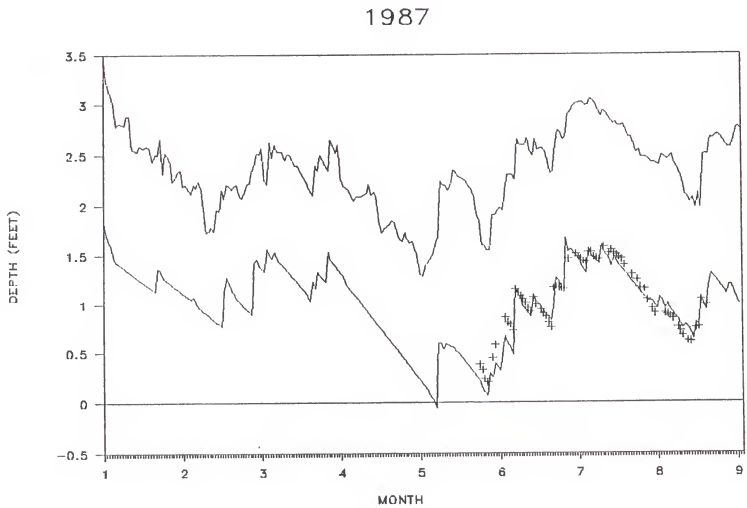



Figure 7-13. A plot showing HYDROMOD-estimated water levels (bottom solid line), daily high water table (top solid line) and staff gauge readings (+) at Dogwood for 1987. Because the lowest elevation of the basin is 1.5 ft MSL, a 1.5 ft discrepancy between HYDROMOD output and water table values is created.

daily high readings, staff gauge water levels and HYDROMOD estimates of water levels. The simulated water levels provide an excellent fit to the water table trend depicted by the well and to the water level values from the staff gauge. Additionally, the basin drydown in early May was verified by field observations. The excellent fit of the estimates to the staff gauge readings also holds credence statistically. The mean $\pm$ SD of the absolute difference between the model and the staff gauge water levels was 0.071 $\pm$ 0.060 ft; the correlation of predicted to actual water levels was 0.968. The mean difference of model - staff gauge water level was -0.028, reflecting a slight underestimate of water level by the model. The mean water level ($\pm$ SD) for the model and concurrent field measurements was 1.062 $\pm$ 0.345 and 1.039 $\pm$ 0.359 ft MSL, respectively.

Graphically, the model preformed poorest in late May - early June when predicted water levels are ca. 0.20 ft below actual levels. Perhaps water puddles on the dry basin, resulting in higher water levels for initial wet season rains. Nonetheless, the accuracy is within the resolution of TAENISIM (strata resolved at 0.20 ft level) to predict oviposition distribution and hatching. Also encouraging is that despite the relatively poor performance in June, model validity became stronger as the season

progressed; perpetuation and subsequent amplification of error(s) did not occur.

Simulation runs of Dogwood water levels in 1985 (Fig. 7-14) and 1986 (Fig. 7-15) also support the validity and application of HYDROMOD. Field observations of drydown and spillover events generally corresponded with predicted water levels. The 1985 run illustrates the advantages of the rain overrun and basin spillover routines in damping water level increases from extreme rain or tide events. The usefulness of the DMOD "floating" water table is shown when the water table does not decline to extreme lows despite extensive drought.

## Discussion

The development of HYDROMOD demonstrated that a few topographical and hydrological characteristics can be used to simulate the complex dynamics of mangrove basin water levels. Geographically, Dogwood's topography was relatively complex due to the increased surface area around red mangrove hummocks. Nevertheless, a simple exponential function was found to provide a valid estimate of the stage-cumulative area relationship for the basin; this function may serve as a characteristic equation whereby stage-area relationships for a basin can be determined by simply measuring basin elevational range and area. Accurate measurements of the elevational range and basin

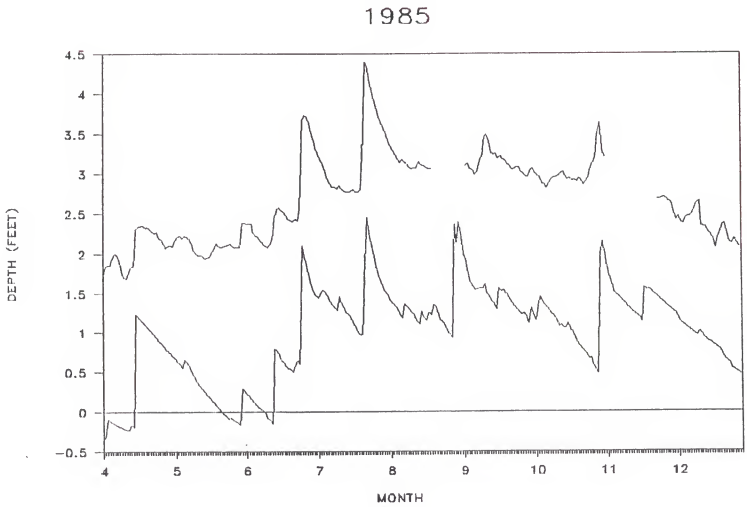



Figure 7-14. A plot showing HYDROMOD-estimated water levels at Dogwood for 1985.

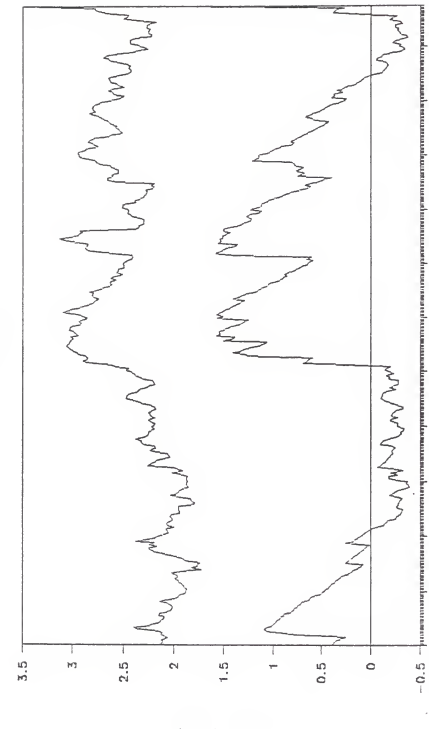



Figure 7-15. A plot showing HYDROMOD-estimated water levels at Dogwood for 1986.

minimum and maximum are necessary for hydrologic model construction.

The inability of the water table wells to map water level at Dogwood and April suggests that well placement is critical if water level measurements are needed, especially in tidal area. Fortunately, well data can be supplemented inexpensively by staff gauges and, if valid, by model estimates. Nonetheless, the well data, supplemented with tide gauge data, were invaluable in depicting the relationship of tide to water table when the basin was dry and when the basin was inundated tidally.

The use of staff gauge water level data provided an economical and powerful tool in developing HYDROMOD. Characteristic ETI rates and the relationship of rainfall, tide and basin topography to changes in water level can be estimated with staff gauge readings. The use of an electronic spreadsheet facilitated the development an construction of HYDROMOD. Model components can be edited easily by using graphical and statistical summaries of model output. This makes curve fitting simple and scenario testing rapid and more comprehendable.

HYDROMOD has several applications to this project and to mosquito ecology and control. HYDROMOD was used to correct invalid well records by adjusting for limitations of the wells and by estimating values for missing data. HYDROMOD was used to generate water levels for input into

TAENISIM to predict Ae. taeniorhynchus populations. This practice can be extended to predict what rain/tide scenarios might produce highest or lowest mosquito populations. Furthermore, hydrology models can be adapted to different mosquito-producing basins to predict mosquito populations over a larger area and at diverse basin types. Basin specific hydrology models might be used to predict water levels both of basin which are frequently flooded by tides, and of basins which are rarely flooded by tides. The use of these coupled-system models could greatly aid in our understanding of Ae. taeniorhynchus population dynamics.

However, before such adventuresome model games are undertaken, the hydrological and entomological validity of both models must be examined. Concerning HYDROMOD, additional and diverse basins must be targeted for modelling. Basins must be characterized and models developed and characterized. Additional detail, such as stage-cumulative pond area, would facilitate mosquito modelling (Rowan et al. 1988). Eventually, a collection of "off the shelf" models might be available to simulate the hydrology of a broad range of mosquito-producing habitats.

# CHAPTER 8
# VERIFICATION OF A SIMULATION MODEL OF AEDES TAENIORHYNCHUS

## Introduction

Validation of simulation models involves three steps (Montague et al. 1982). First, the overall pattern produced by predicted populations is compared to observed patterns in nature. Second, sensitivity analysis of model parameters is performed to indicate if the model behaves normally within the conceivable range of parameter values (e.g. survival rates, egg clutch size). Third, model output is compared to data from sampled field and/or laboratory populations; this is termed model verification. The output pattern and sensitivity analysis for TAENISIM was presented in Ch. 6; this chapter will concern itself with model verification.

Verification methodology, while nonstandard, reflects the objectives and applications of the model. If the model was designed to provide accurate estimates of populations for a fixed site during a specific time, verification would involve obtaining valid population estimates at that site during that time period. Conversely, if the model was designed only to predict species presence/absence, verification would only involve the sampling necessary to determine species presence/absence. In models of mosquito population dynamics, Haile and Weidhaas (1977) compared

relative changes in predicted female Anopheles albimanus Wiedemann populations to field collections from man-biting collections and horse-baited traps. Focks et al. (1988b) verified a simulation model of Psorophora columbiae by comparing the relative size and time of occurrence for predicted and field estimated larval and adult populations; predicted egg densities were compared to egg density ranges reported in the literature.

The objective of TAENISIM, at this point, was to see if the hypothesis concerning Aedes taeniorhynchus population dynamics (see Ch. 6) approximates natural populations. Thus, validation involving the output pattern and sensitivity analysis was largely presented in Ch. 6. The objective of this chapter is to validate TAENISIM by examining simulated population dynamics for abnormal behavior and by comparing relative changes in predicted egg and larval populations to relative changes in field populations at Dogwood.

## Materials and Methods

### Premise for Model Validation

Model validation consisted of three stages. First, population estimates of Ae. taeniorhynchus egg and larval populations were made from samples taken at Dogwood during 1985-1987. Rainfall and tide data were collected and used to calculate Dogwood water level at 24-hr intervals using

HYDROMOD (see Ch. 7). Second, the HYDROMOD water level estimates and rain and tide date were input into TAENISIM to produce yearly simulations of Ae. taeniorhynchus populations for 1985-1987. Third, TAENISIM was verified by examining model output for unrealistic behavior (i.e. unrealistic model predictions) and comparing model output to relative egg, larval and adult populations sampled at Dogwood.

## Verification of TAENISIM

### Simulation Runs

Simulation runs for 1985-1987 were conducted and the model output examined for "unrealistic behavior". The model was initialized as follows: 148 eggs were allocated for the eight respective MATVIABLEGG strata (strata 1 - 8) as follows: 5, 20, 50, 30, 20, 10 and 8. Adult females were initialized at 5, a value arbitrarily thought to represent moderate populations. At week 18 (early May) an influx of 20 females, representing a spring migration often observed at Dogwood, was input into the model. These mosquitoes produced ca. 300 eggs over all strata, or 300/8 = 37.5 eggs/sample, a typical egg density encountered in late May. The model was run using QUICKBASIC® on an IBM AT® computer. Output was stored as an ASCII file that was imported into LOTUS 123® for graphing. Runs were initialized each year beginning on 1 April 1985 and ending on 1 September 1987.

Simulation runs were examined for realistic behavior as an initial step in debugging and validation. Unusual behavior, such as sudden population fluctuations, extinction and missing phenological events (e.g. adult emergence) was sought out and used to guide model debugging. Initial simulation runs exposed model errors that were corrected before final verification runs. The most significant change involved expanding the level equations for immature and mature eggs (IMMVIABLEGG and MATVIABLEGG in TAENISIM) from a 1 X 8 to a 3 X 8 array. This was necessary for simultaneous aging of up to three cohorts of eggs laid by overlapping broods. Unfortunately, the additional calculations increased significantly the calculation time for the model.

Verification using Estimated Populations at Dogwood

Egg populations

Absolute population estimates were obtained using an area sampler and a systematic sampling procedure (see Ch. 2 for details). Samples were collected during 1986 and 1987. Since elevations of individual samples were known, the stage-specific egg population was calculated and used to determine the overall population. Stages were defined by the criteria used in TAENISIM in which stage 1 represents elevations < 1.8 feet (0.54 m) MSL; subsequent stages are stepped at 0.20 ft (0.06 m) increments. Stage 8,

representing elevations above the basin spillover elevation, encompasses elevations 3.0 - 3.6 ft (0.9 - 1.08 m) MSL. Samples were collected systematically along elevation contours depicted on a topographic map (Ch. 7) and eggs per sample estimated by flooding samples and counting larvae 24-hr later.

Calculation of the mean egg population estimate used to verify TAENISIM output involved several steps. First, the mean egg population per stage was calculated. This value was then multiplied by the relative area of the stage; the following rationale was used to develop the procedure. If the strata were of equal area, each would contain 1/8 or 12.5% of total basin area and the mean population of eggs for the entire basin would simply be the arithmetic mean of strata populations. However, strata are not the same size; for example, stage 8 encompasses ca. 0.13 ha or 18.7% of the Dogwood basin. A relative area correction factor was developed for each stratum from the stage-area relationship developed in Ch. 7. For example, the stage correction factor for stage 8 would be 18.7%/12.5% = 1.5. The stage-specific egg population was multiplied by the area correction factor then summed over all stages to obtain the total population.

Model estimates of egg populations were based on a perceived egg population for a 10-cm diameter core. Egg populations (consisting only of mature, viable eggs; variable MATVIABLEGG in TAENISIM) were summed over the eight elevational strata.

Larval Populations

Two methods were used to sample larvae. Four populations (1985) were sampled with an area sampler while other populations were sampled with a plastic dipper. The area sampler consisted of a 0.7-m, 36-cm diam. galvanized metal cylinder that enclosed an area of 0.10-$m^2$; the dipper was 10-cm. in diameter, enclosing an area of 0.008-$m^2$ with a capacity of 350-ml. While the dipper is not an area sampler, population estimates of Aedes larvae using a dipper and an area sampler have been correlated positively (Crosen et al. 1976, O'Meara et al. 1988). Area sampler data were calibrated to dipper data by multiplying the area sampler estimate by the ratio of the area of the dipper to the area sampler (0.008/0.10 = 0.08). The area sampler was abandoned as a larval sampler because only the dipper could effectively sample larvae in shallow, vegetation-clogged water.

Larval populations were estimated from a stratified sampling procedure within the Dogwood grid (see Ch. 7 for details of grid topography). Larval populations at Dogwood were distributed according to basin flooding

history. Larvae were distributed throughout the flooded basin when the basin was primarily dry before flooding. Larvae were concentrated along margins when a hatch occurred following additional flooding of a previously flooded basin. In extreme cases, larvae were limited to isolated puddles when previously flooded basins contained high fish populations.

A stratified sampling program was established to take advantage of these distributions. Newly and previously flooded areas should have distinct larval populations and, thus, could serve as sampling strata (Cochran 1963). Strata were defined as newly flooded (grid contained some exposed land before flooding event) and previously flooded (entire grid was covered with water 0.5+ ft (0.15+ m) deep prior to new flooding event). Since the grid was inspected at < 48-hr intervals, flooding history was well known.

Larval sampling and population estimates were conducted as follows. Either one (area sampler) or five (dipper) samples were taken for each flooded grid. Larvae in the area sampler were collected with a fine-mesh minnow net and counted in a enamel pan; sampling continued until no larvae were collected. Sampling efficiency using known numbers of 2nd instar larvae was 93%. Larvae within the dipper were counted by slowly pouring water from the dipper. The mean number of larvae per sample was calculated for newly and previously flooded grids. Sampling was usually

conducted within 48 hr of hatch; most data reflect populations of 1st and 2nd larval instars.

The mean number of larvae per dip for the basin was calculated using the stage-cumulative area relationship described in Ch. 7. With this relationship, the proportion of area flooded before and after a flooding event could be calculated and used to partition the basin into newly and previously flooded areas. For example, if a rain increased area flooded from 0.40 to 0.60 ha, the proportion newly flooded would be (0.60-0.40)/0.60 = 0.33. This value was used to weigh larval population estimates from newly and previously flooded areas in calculating a pooled estimate of the mean number of larvae per dip for the entire basin. If, in our example, the respective mean number of larvae per dip was 10 and 1 for newly and previously flooded areas, the pooled estimate would be (10 X 0.33) + (1 X 0.67) = 3.97 larvae per dip.

Since natural populations of larvae had undoubtedly suffered some mortality before sampling, calculation of larval populations in TAENISIM incorporated larval mortality. These were expressed as the product of the proportion of larvae surviving (1 - LARVMORT in TAENISIM), a correction factor and the number of eggs hatching. A correction factor of 0.80 was arbitrarily chosen to account for disproportionate mortality in the 1st two larval instars (Lakhani and Service 1974).

## Results

### Simulation Runs-General Behavior

The simulation runs indicate that TAENISIM appears to produce a realistic pattern of population dynamics for egg, larvae and adult female populations. Egg populations (Fig. 8-1) peaked in May and June for 1985 and 1986 following oviposition by migrants. Egg populations peaked in August for the 1987 simulation run; clearly, egg populations were highest during 1987. Larval and adult female populations (Fig. 8-2 and 8-3, respectively) also exhibited realistic dynamics; highest populations and greatest number of broods were found in the summer. Also, highest populations were found in 1987. The relatively high January populations reflect the egg populations used to initialize the model. Perhaps actual winter populations are lower.

### Model Verification

#### Egg populations

Egg population estimates from TAENISIM appeared to conform with relative populations estimated from field samples. During 1986, predicted and field-estimated egg populations exhibited an initial high peak in May followed by a crash leading to a series of small peaks for the remainder of the summer (Fig. 8-4). However, the October field estimate (14.81 eggs) was considerably higher than the model had predicted. This reflects the tendency for

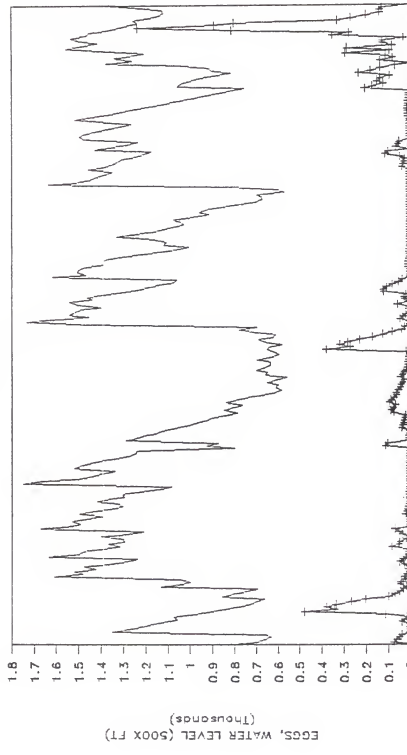



Figure 8-1. A plot depicting relative Aedes taeniorhynchus egg populations at Dogwood estimated by TAENISIM. HYDROMOD-estimated water levels provided to show relationship between the two variables.

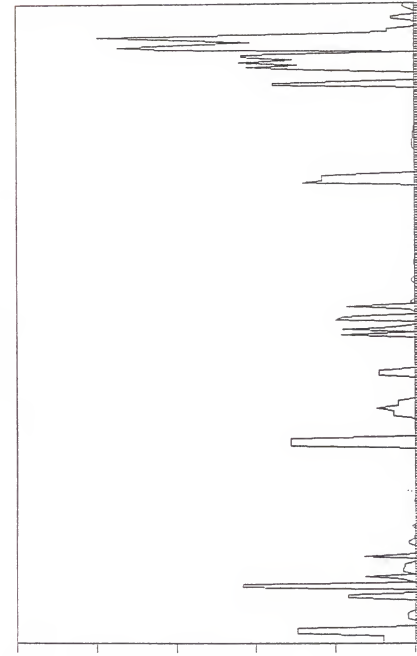



Figure 8-2. A plot depicting relative Aedes taeniorhynchus larval populations at Dogwood estimated by TAENISIM.

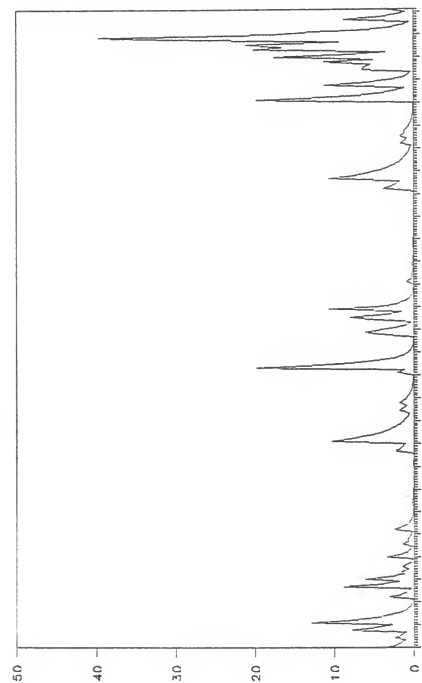



Figure 8-3. A plot depicting relative Aedes taeniorhynchus adult female populations at Dogwood estimated by TAENISIM.

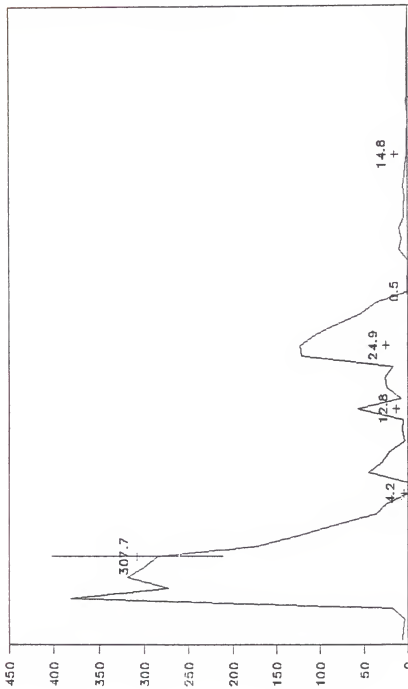



Figure 8-4. A plot showing egg populations estimated by TAENISIM and estimated from field samples (mean and SE given) at Dogwood for 1986.

model populations to crash toward the end of summer. Results for 1987 (Fig. 8-5) were limited to two field estimates; nonetheless, the model dramatically underestimated the persistence of the winter egg populations evidenced by the late March sample. Without additional field estimates, it is difficult to assess the significance of the late May estimate. Interestingly, the early August egg populations were the largest values predicted by TAENISIM. Field observations of larval broods in August suggest that a large egg population was present at this time.

Larval populations

Results for the stratified systematic sampling program indicate that previously flooded grids contained significantly lower larval populations than newly did flooded grids. Dipper counts for newly vs previously flooded grids were significantly different ($P < 0.01$, $X^2$ test (Schlotzhauer and Littell 1987)) for all but one of the nine margin and puddle-distributed larval populations sampled. Table 8-1 shows a summary of the distribution of larvae for these conditions. Since the relationship between stage and puddle area is unknown, calculation of mean larval per dip for the basin could not be done accurately for the two puddle broods. For purposes of model verification, however, overall mean larvae per dipper

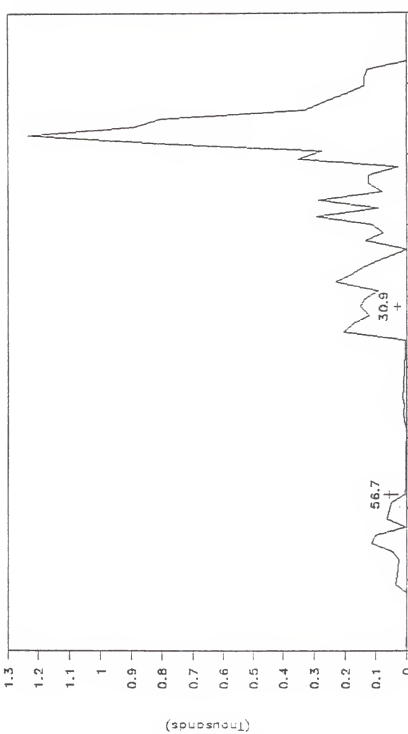



Figure 8-5. A plot showing egg populations estimated by TAENISIM and estimated from field samples (mean and SE given) at Dogwood for 1987.

was estimated; puddles were assumed to make up only 5% of flooded basin area.

Table 8-1. Aedes taeniorhynchus larval populations at Dogwood (1986-1987); data is mean number of larvae per dip.

| Larval Distribution | Reps | Grid flooding history Newly | Previously | Overall |
|---|---|---|---|---|
| A. Extensive | 5 | 5.97 | - | 5.97 |
| B. Marginal | 7 | 2.39 | 0.02 | 0.98 |
| C. Puddle | 2 | 9.34 | 0 | ? |

Field estimates of larval populations indicate that TAENISIM accurately simulates the timing of larval hatching but that predicted population size may be inaccurate. The model performed well during 1985 (Fig. 8-6), with the timing and relative size of larval populations in agreement with field data. Again in 1986 (Fig. 8-7), predicted brood timing was in agreement with field data. Performance of the model to predict population size in 1986 is difficult to assess, although the model and field populations are clearly the lowest of the three years.

The model performance in predicting larval populations in 1987 (Fig. 8-8) is somewhat misleading. First, not all broods hatched were sampled due to time constraints. Second, mosquito control personnel began adulticiding the Dogwood site using helicopters. Observations suggest that

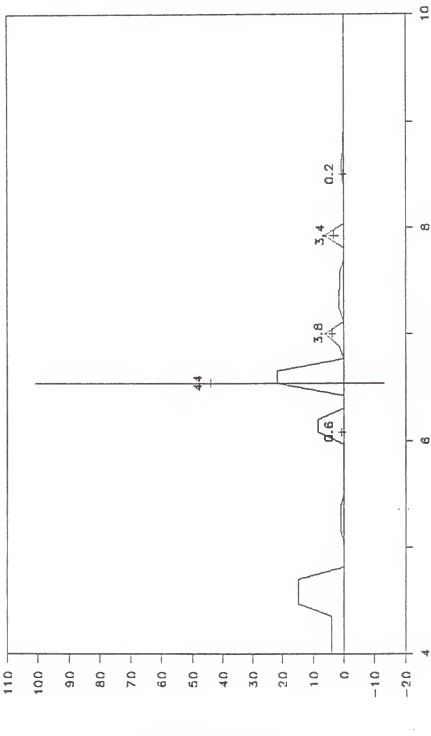



Figure 8-6. A plot showing larval populations estimated by TAENISIM and estimated from field samples (mean and SE given) at Dogwood for 1985.

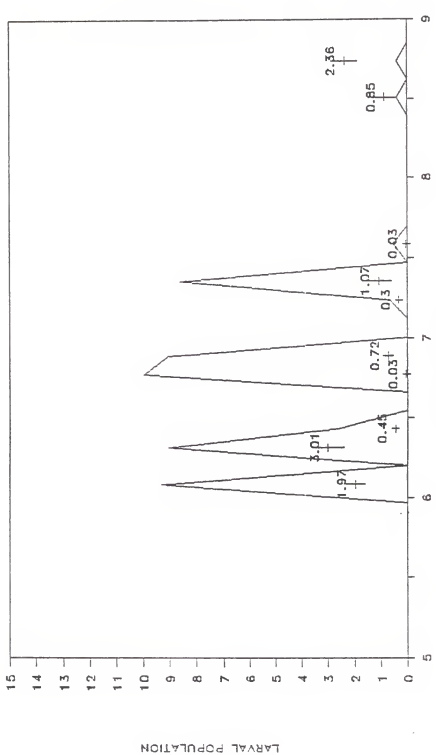



Figure 8-7. A plot showing larval populations estimated by TAENISIM and estimated from field samples (mean and SE given) at Dogwood for 1986.

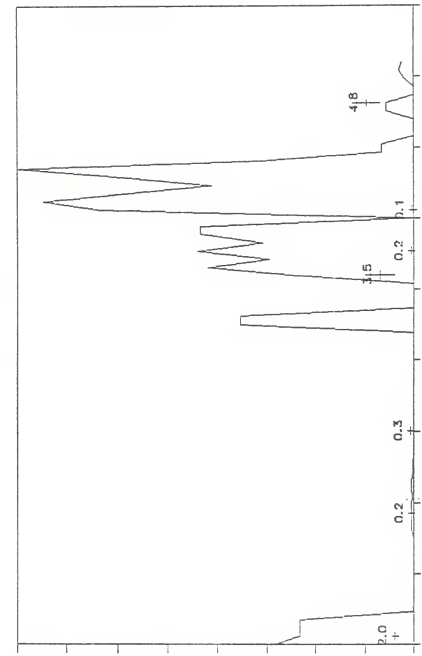



Figure 8-8. A plot showing larval populations estimated by TAENISIM and estimated from field samples (mean and SE given) at Dogwood for 1987.

the helicopter downdraft enhances insecticide penetration such that adulticides may kill early instar larvae.

The model appeared to have missed the prediction of the largest brood sampled during the course of the study (August 1987). Apparently, the model missed predicting a major larval population by a relatively short time period. Figure 8-1 shows that the highest egg populations predicted by the model for all runs were in early August 1987. However, by the time flooding occurred, oviposition had nearly ceased and a majority of the eggs had been killed. The large hatch in late August indicates that either oviposition or high egg populations persisted longer than predicted by TAENISIM. Daily mosquito collections (predominant species was Ae. taeniorhynchus) from a New Jersey light trap located ca. 3 km from Dogwood indicated that a large influx of Ae. taeniorhynchus occurred in early July. From this, oviposition and mature egg populations could be expected to peak in mid to late July; this corresponds well to model estimates for egg populations at Dogwood (Fig. 8-1). The results suggest, however, that the subsequent crash in egg populations predicted for the first half of August was overestimated. This might have been due to the presence of immigrating gravid mosquitoes that maintained high oviposition rates well into August.

## Discussion

While verification suggests that TAENISIM realistically simulates Ae. taeniorhynchus population trends, several problems are apparent. Verification was greatly complicated by the confounding impact of adult migration and pesticide application on mosquito populations. Ultimately, model verification should be conducted on sites isolated from migrants and mosquito control activity. However, the Dogwood site does represent typical Ae. taeniorhynchus habitat in the Collier Mosquito Control District. Thus, model performance under less than ideal circumstances was encouraging.

Despite these problems, the verification procedure elucidated several interesting aspects of TAENISIM. Most of the model "failures" (i.e., poor agreement with field estimates) resulted from rapid mortality of eggs, larvae and adult females. The tendency for the model to "go to extinction" in late summer elucidates the impact that larval predation and lack of migration have on Ae. taeniorhynchus population dynamics. Perhaps this mosquito migrates to oviposit in basins that are drier and contain low fish populations in order to minimize larval mortality. With asynchronous fluctuations in environmental quality, dispersal offers some chance to encounter better conditions elsewhere (Gadgil 1971). This is apparently what occurs in southwest Florida during late spring migrations of Ae.

taeniorhynchus adults that hatched via tidal flooding. Tidally-flooded basins are often infiltrated with high populations of poecilid fish within weeks of flooding (Ch. 6). Migration to and oviposition in basins not flooded by the tide (such as Dogwood) enhances the chance that larvae will survive. It is also interesting that the input of the May migrants served to fuel the model throughout the summer.

The benefits of a simulation model such as TAENISIM go beyond the ability of the model to predict populations accurately. As witnessed, simulation models provide excellent tools to examine causal hypotheses concerning population dynamics. They serve to highlight gaps in knowledge, data and research. From this project, it is clear that additional research on stage-specific mortality, the interaction of flooding duration and fish populations, and the dynamics of Ae. taeniorhynchus dispersal would greatly enhance the validity of TAENISIM.

CHAPTER 9
DISCUSSION

## Introduction

This discussion will center on four themes (chapter subheadings will be enclosed in parentheses). First, because this project represents an intial step in a series of research projects designed to develop methods to simulate Ae. taeniorhynchus population dynamics in southwest Florida, an assessment of the information and methods that are ready for use in the model is needed (STATUS REPORT). Second, problems that need immediate attention must to be identified and prioritized (SHORT-TERM OBJECTIVES). Third, the key elements necessary to develop and validate a large-scale (i.e. spatial) simulation of mosquito production in a variety of mangrove habitats will be identified (KEY ELEMENTS FOR LARGE-SCALE MODEL CONSTRUCTION AND VALIDATION). Fourth, tentative applications and future research plans resulting from this project will be presented (LONG-TERM OBJECTIVES).

## Status Report

Currently, this project has provided valuable information and a design for the development of regional mosquito population simulation models. The sampling methods, (eggs and larvae), many of the egg-associated

rates and the conceptualization and construction processes for the hydrological and mosquito population models are in place. The information listed below represents data that are valid generalizations for use in a simulation model. Additional work on these subjects would probably result in diminishing returns for work effort spent, detracting from progress in more critical areas.

Much of the information concerning the mosquito life cycle has been studied rigorously and is ready for application in a simulation model. The general egg distribution pattern, hatching percentage, clutch size, fertility and sampling procedure appears valid. Most of the larval and adult Ae. taeniorhynchus information was obtained from the literature and is presumed valid. The general construction procedure for TAENISIM, whereby sites are stratified by elevation, proved successful in simulating mosquito oviposition and hatching realistically. The model can be used as is, although improvements in computational efficiency and "user friendliness" are needed. The procedure for obtaining estimates of the hydrological parameters used to construct a basin hydrology model is in place. Staff gauges proved more effective and cost effective that did water table wells. The use of a computer spreadsheet greatly facilitated model construction.

Short-term Objectives

Despite the potential applications of this project, several problems exist that deserve immediate attention. Field-estimated egg predation rates are suspiciously high. The leaf test, although elegant, may make eggs more accessible than anturally occurring eggs to predators. Since oviposition site and clutch size may have a significant impact on predation rates (Subinprasert and Svensson 1988), it is important to validate and calibrate leaf and ring test results with more realistic estimates of egg predation. Simultaneous estimates of predator populations would help to verify if predation of mosquito eggs is density dependent. Additional work needs to be done at other sites to validate oviposition preference for red mangrove litter; this information will be used to select sites for further model validation. Finally, the egg sampling procedure, albeit valid, is very labor intensive. Since the objective of TAENISIM is to predict population trends, relative population estimates involving less intensive sampling should suffice. The egg sampling data from this study could be used to develop a sequential sampling procedure (Regniere et al. 1983) that would reduce sample size and streamline the verification procedure.

The reality and efficiency of TAENISIM can be increased markedly by upgrading a few critical attributes.

TAENISIM simulation runs were slow, especially after the expansion of egg level arrays. Computation speed could be increased by using IF statements and subroutines that bypass calculations unless absolutely neccessary. Also, a small elevational range version, incorporating fewer elevational strata, would increase computation speed. This version would be applicable to shallow basins such as April. Substituting field data for simulated temperatures should also increase model computation speed while improving the reality of temperature-dependent rates.

Modifications are also needed for HYDROMOD. The exponential stage-area relationship and the hydrological parameters used in HYDROMOD must be examined at other basins; hopefully, general estimates can be validated. The impact of stage on the relative change in water level due to rain needs to be incorporated in HYDROMOD; clearly, the rate of increase of water level should decrease as the cumulative area flooded increases for a fixed volume input of water. The failure to incorporate this relationship in HYDROMOD would account for the underestimates of June 1987 water levels (Fig. 7-13). This relationship could be added easily to HYDROMOD using the stage-area relationship for the basin. Also, the stage-area relationship needs to be updated to account for the area of isolated ponds critical to mosquito production (Rowan et al. 1988).

## Key Elements for Large-scale Model Construction and Validation

As mentioned in the introduction, the objective of this project was to identify the key information needed to develop large-scale (geographical) simulation models of salt marsh mosquito production. This project has initiated a definition of the important hydrological and biological information needed for model construction. Hydrological models require both basin-specific and generalized hydrological parameters. Basin-specific parameters to be measured are the elevational range, general surface contour (slope, presence of tussocks and isolated ponds), stage-area relationship and spillover elevation. However, the specific stage-area relationship can be estimated from the elevational range and general topography using the exponential function derived in Ch. 7. General estimates of ETI and the relationships between water level and rain and tide might be applicable to all or a class of basins.

Not surprisingly, identification of the biological information needed to develop a large-scale simulation model has been more difficult. While TAENISIM may currently produce realistic output, incorporation of the following suggestions should increase the "reality" of the model. The validity of each stage-specific mortality rate, shown by sensitivity analysis (Ch. 6) to have a profound impact on model predictions, should be closely examined.

Personal observations and the literature indicate that the relationship of fish density to larval mortality (Meisch 1985), and of pesticides to all stage-specific mortality rates (Haile and Weidhaas 1979, Hurlbert et al. 1972), are of particular importance. The role of egg flushing and runoff hatching at different sites deserve attention. Adult migration should be incorporated into the model (Wilkerson et al. 1986). Model initialization becomes critical as different sites are modeled. Site-specific mosquito population estimates from a variety of sites are needed in order to initialize population levels properly in a large-scale simulation model. A sequential sampling program for eggs will greatly facilitate these efforts. And finally, the model must be readily accessible to users. Development of better graphics and user-friendly subroutines that allow the user to input hydrological scenarios and mosquito populations, and to change parameter values, would greatly enhance the utility of TAENISIM.

Ultimately, the coupled HYDROMOD-TAENISIM model must be validated for other sites. A range of salt marsh mosquito producing sites must be tested. This can be accomplished with a minimum of sampling by using the wealth of historical data available from Collier Mosquito Control District to complement intensive sampling at a few select sites.

Long-term Objectives

The ambitious projects outlined above require a great deal of effort, time and money before they are anything more than conjecture. Nonetheless, the culmination of these ideas deserve our attention and speculation. The following represent projects that would improve the model and/or benefit from the model. As previously mentioned, fish are an important element in TAENISIM.

*Development of a hydrologically-based fish model would greatly increase the realism of TAENISIM (although at a loss of computation speed!).

*A salt marsh mosquito simulation model could be used to identify worst and best case meteorological scenarios so that these scenarios could be used to anticipate mosquito production.

*The model could be used to develop spatial migration models and to test hypotheses concerning migration.

*The model could be used to optimize IPM programs (Focks et al. 1988, Haile and Weidhaas 1977) for the control of Ae. taeniorhynchus.

*The model could be used to identify key stages in the mosquito life cycle that are most responsible for population growth.

*The model could serve as a template to develop models for other mosquitoes and animals occupying a similar niche.

*The model could be coupled to other models (weather, fish, financial, etc.) in order to study and predict a range of mosquito-related phenomena.

And, finally, the model has several practical applications for mosquito control.

*The model allows monitoring of sites that are not accessible by personnel.

*Mosquito population trends could be predicted well in advance or simply updated based on current weather data.

*The model can temporally and spatially indentify areas most likely to produce mosquitoes and can suggest optimal treatment scheduling.

*Model output could be used in a dynamic mapping program (Taylor 1985) to visualize the spatial distribution of mosquito populations and coordinate control activities. Certainly there are many other potential applications of the model. Ultimately, application of the model depends upon proof of validity; a great deal of work remains to be done. But at the very least, the model does serve as a tool for better understanding of the dynamics of the mangrove-Ae. taeniorhynchus system and to spotlight research needs.

APPENDIX A

SUMMARIZED STATISTICS FOR AEDES TAENIORHYNCHUS EGG POPULATIONS

I. Lowland substrate at Dogwood

| Date | N | Mean | SD | k |
|---|---|---|---|---|
| 6 Nov. 1984 | 160 | 39.9 | 70.5 | 0.20 |
| 30 May 1986 | 80 | 12.7 | 23.1 | 0.11 |
| 3 June 1986 | 40 | 62.9 | 180.0 | 0.19 |
| 12 Aug. 1986 | 30 | 12.4 | 22.0 | 0.25 |
| 12 Oct. 1986 | 58 | 13.5 | 70.5 | 0.10 |
| 7 Mar. 1987 | 48 | 30.7 | 36.4 | 0.73 |
| 9 June 1987 | 76 | 4.3 | 10.5 | 0.17 |

II. Upland substrate at Dogwood

| Date | N | Mean | SD | k |
|---|---|---|---|---|
| 6 Nov. 1984 | 80 | 11.4 | 22.8 | 0.16 |
| 16-18 June 1986 | 161 | 2.8 | 6.2 | 0.12 |
| 14-16 July 1986 | 102 | 13.2 | 17.2 | 0.34 |
| 17 July 1986 | 53 | 0.6 | 2.2 | 0.10 |
| 12 Aug. 1986 | 32 | 1.8 | 4.0 | 0.14 |
| 21 Aug. 1986 | 110 | 0.5 | 2.0 | 0.08 |
| 12 Oct. 1986 | 40 | 1.6 | 7.1 | 0.06 |
| 7 Mar. 1987 | 48 | 0.6 | 2.0 | 0.09 |
| 9 June 1987 | 20 | 0.2 | 0.6 | ND |

APPENDIX B

DATA COLLECTED FROM THE APRIL GRID ON 28 JULY 1987.

Data (n = 100) are defined as follows:

| | | |
|---|---|---|
| X and Y | = | grid coordinates in meters |
| eggs | = | number of Aedes taeniorhynchus eggs/sample |
| log egg | = | log (egg + 1) |
| snail | = | number Melampus coffeus/sample |
| wt | = | depth to water table (in cm) at X,Y |
| black | = | number of black mangrove leaves >50% intact per sample |
| red | = | number of red mangrove leaves >50% intact per sample |

| X | Y | eggs | log egg | snail | wt | black | red |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 27 | 0 | 2 |
| 0 | 1 | 0 | 0 | 0 | 26.5 | 0 | 1 |
| 0 | 2 | 0 | 0 | 2 | 25.5 | 0 | 1 |
| 0 | 3 | 0 | 0 | 3 | 24.5 | 1 | 1 |
| 0 | 4 | 0 | 0 | 5 | 22.5 | 0 | 1 |
| 0 | 5 | 0 | 0 | 1 | 20.5 | 0 | 0 |
| 0 | 6 | 0 | 0 | 2 | 19 | 0 | 1 |
| 0 | 7 | 0 | 0 | 0 | 18 | 0 | 2 |
| 0 | 8 | 0 | 0 | 2 | 17.5 | 1 | 2 |
| 0 | 9 | 0 | 0 | 2 | 15 | 1 | 2 |
| 6 | 0 | 0 | 0 | 1 | 21 | 0 | 0 |
| 6 | 1 | 0 | 0 | 3 | 22.5 | 0 | 1 |
| 6 | 2 | 1 | 0.30102 | 2 | 21 | 1 | 1 |
| 6 | 3 | 1 | 0.30102 | 1 | 18 | 0 | 4 |
| 6 | 4 | 0 | 0 | 1 | 17 | 0 | 6 |
| 6 | 5 | 2 | 0.47712 | 3 | 17 | 0 | 6 |
| 6 | 6 | 6 | 0.84509 | 0 | 15.5 | 1 | 4 |
| 6 | 7 | 2 | 0.47712 | 3 | 15 | 1 | 6 |
| 6 | 8 | 29 | 1.47712 | 0 | 15 | 1 | 9 |
| 6 | 9 | 0 | 0 | 0 | 12.5 | 1 | 8 |
| 12 | 0 | 0 | 0 | 2 | 17 | 3 | 3 |
| 12 | 1 | 0 | 0 | 1 | 15.5 | 1 | 1 |
| 12 | 2 | 1 | 0.30102 | 6 | 16.5 | 1 | 2 |
| 12 | 3 | 0 | 0 | 1 | 14.5 | 1 | 2 |
| 12 | 4 | 40 | 1.61278 | 0 | 12 | 3 | 6 |
| 12 | 5 | 8 | 0.95424 | 0 | 10.5 | 7 | 5 |
| 12 | 6 | 0 | 0 | 0 | 10 | 5 | 11 |
| 12 | 7 | 1 | 0.30102 | 0 | 13 | 10 | 6 |
| 12 | 8 | 10 | 1.04139 | 0 | 12 | 7 | 7 |
| 12 | 9 | 37 | 1.57978 | 0 | 12.5 | 2 | 10 |

| x | y | eggs | log egg | snail | wt | black | red |
|---|---|---|---|---|---|---|---|
| 18 | 0 | 0 | 0 | 0 | 19.5 | 5 | 6 |
| 18 | 1 | 7 | 0.90308 | 0 | 13.5 | 2 | 6 |
| 18 | 2 | 27 | 1.44715 | 0 | 10.5 | 4 | 2 |
| 18 | 3 | 3 | 0.60205 | 0 | 18 | 7 | 10 |
| 18 | 4 | 0 | 0 | 0 | 17 | 5 | 7 |
| 18 | 5 | 7 | 0.90308 | 0 | 6.5 | 3 | 3 |
| 18 | 6 | 0 | 0 | 0 | 7 | 2 | 7 |
| 18 | 7 | 0 | 0 | 0 | 7 | 1 | 9 |
| 18 | 8 | 0 | 0 | 0 | 8 | 4 | 9 |
| 18 | 9 | 0 | 0 | 0 | 8 | 2 | 10 |
| 24 | 0 | 0 | 0 | 0 | 7 | 5 | 11 |
| 24 | 1 | 0 | 0 | 0 | 6 | 2 | 6 |
| 24 | 2 | 0 | 0 | 1 | 5.5 | 2 | 2 |
| 24 | 3 | 0 | 0 | 0 | 6 | 2 | 2 |
| 24 | 4 | 0 | 0 | 0 | 7 | 8 | 0 |
| 24 | 5 | 0 | 0 | 0 | 7 | 4 | 0 |
| 24 | 6 | 0 | 0 | 0 | 6.5 | 7 | 1 |
| 24 | 7 | 0 | 0 | 0 | 3.5 | 5 | 0 |
| 24 | 8 | 0 | 0 | 0 | 4 | 10 | 1 |
| 24 | 9 | 0 | 0 | 0 | 4 | 7 | 0 |
| 30 | 0 | 0 | 0 | 0 | 11.5 | 6 | 9 |
| 30 | 1 | 0 | 0 | 1 | 5.5 | 8 | 2 |
| 30 | 2 | 0 | 0 | 0 | 5 | 6 | 1 |
| 30 | 3 | 0 | 0 | 0 | 5.5 | 8 | 0 |
| 30 | 4 | 0 | 0 | 1 | 6.5 | 9 | 0 |
| 30 | 5 | 0 | 0 | 1 | 7.5 | 8 | 0 |
| 30 | 6 | 26 | 1.43136 | 0 | 9.5 | 13 | 1 |
| 30 | 7 | 0 | 0 | 1 | 9 | 22 | 0 |
| 30 | 8 | 0 | 0 | 0 | 9.5 | 3 | 2 |
| 30 | 9 | 0 | 0 | 1 | 10 | 11 | 1 |
| 36 | 0 | 0 | 0 | 0 | 9.5 | 0 | 0 |
| 36 | 1 | 0 | 0 | 0 | 12 | 3 | 0 |
| 36 | 2 | 0 | 0 | 0 | 12 | 4 | 0 |
| 36 | 3 | 0 | 0 | 1 | 11.5 | 8 | 0 |
| 36 | 4 | 0 | 0 | 0 | 11 | 9 | 0 |
| 36 | 5 | 1 | 0.30102 | 0 | 10 | 2 | 0 |
| 36 | 6 | 0 | 0 | 0 | 10 | 3 | 0 |
| 36 | 7 | 0 | 0 | 0 | 9 | 0 | 0 |
| 36 | 8 | 0 | 0 | 0 | 8.5 | 1 | 0 |
| 36 | 9 | 0 | 0 | 0 | 7.5 | 0 | 0 |
| 42 | 0 | 0 | 0 | 5 | 12.5 | 9 | 0 |
| 42 | 1 | 0 | 0 | 1 | 13.5 | 4 | 0 |
| 42 | 2 | 0 | 0 | 0 | 13.5 | 0 | 0 |
| 42 | 3 | 0 | 0 | 0 | 15 | 3 | 0 |
| 42 | 4 | 0 | 0 | 0 | 12.5 | 1 | 0 |
| 42 | 5 | 0 | 0 | 0 | 13.5 | 4 | 0 |
| 42 | 6 | 0 | 0 | 0 | 13.5 | 0 | 0 |
| 42 | 7 | 0 | 0 | 0 | 13 | 0 | 0 |
| 42 | 8 | 0 | 0 | 1 | 13 | 2 | 0 |
| 42 | 9 | 0 | 0 | 1 | 13 | 1 | 0 |

| x | y | eggs | log egg | snail | wt | black | red |
|---|---|---|---|---|---|---|---|
| 48 | 0 | 0 | 0 | 6 | 19 | 4 | 0 |
| 48 | 1 | 0 | 0 | 1 | 19.75 | 1 | 0 |
| 48 | 2 | 0 | 0 | 5 | 19.5 | 1 | 0 |
| 48 | 3 | 0 | 0 | 0 | 19 | 3 | 0 |
| 48 | 4 | 0 | 0 | 5 | 18.5 | 0 | 0 |
| 48 | 5 | 0 | 0 | 3 | 18 | 0 | 0 |
| 48 | 6 | 0 | 0 | 5 | 18 | 3 | 0 |
| 48 | 7 | 0 | 0 | 0 | 17 | 0 | 0 |
| 48 | 8 | 0 | 0 | 0 | 17.5 | 1 | 0 |
| 48 | 9 | 0 | 0 | 3 | 19 | 0 | 0 |
| 54 | 0 | 0 | 0 | 3 | 23 | 3 | 0 |
| 54 | 1 | 0 | 0 | 0 | 24 | 0 | 0 |
| 54 | 2 | 0 | 0 | 1 | 24 | 0 | 0 |
| 54 | 3 | 0 | 0 | 0 | 24.25 | 3 | 0 |
| 54 | 4 | 0 | 0 | 14 | 24.5 | 0 | 0 |
| 54 | 5 | 0 | 0 | 0 | 23.5 | 2 | 0 |
| 54 | 6 | 0 | 0 | 2 | 23 | 3 | 0 |
| 54 | 7 | 0 | 0 | 7 | 23 | 1 | 0 |
| 54 | 8 | 0 | 0 | 2 | 23.5 | 0 | 0 |
| 54 | 9 | 0 | 0 | 3 | 23 | 1 | 0 |

## APPENDIX C
## SAMPLE CALCULATIONS FROM EGG MORTALITY STUDIES

Sample values of weekly egg mortality from the ring test calculated according to two methods. Eggs were placed in the field on 21 Feb. 1987 and retrieved on 7 March 1987 for a two week exposure period. Ten eggs per test ring with 15 test and 5 control (no eggs added) rings. Rings were flooded after retrieval; a mean of 5.27 $\pm$ 3.65 (SD) eggs/ring and 4.67 $\pm$ 3.67 larvae/ring were found.

Method A. Larval hatching

1. Calculate EXPECTED MEAN NUMBER OF LARVAE/RING = ORIGINAL EGGS/RING X FERTILITY X HATCH X LARV. SURVIVAL
   10 X 0.925 X 0.925 = 8.35 larvae/ring.

2. Calculate EGGSURVIVAL
   EGGSURVIVAL = MEAN NUMBER LARVAE/RING (TEST) divided by EXPECTED MEAN NUMBER OF LARVAE/RING =
   4.67/8.35 = 0.56

3. Calculate WEEKLY MORTALITY RATE
   WEEKLY MORTALITY RATE = 1 - WEEKLY SURVIVAL RATE
   WEEKLY SURVIVAL RATE = EGGSURVIVAL raised to the 1/T power where T is the exposure period in weeks.

   WEEKLY SURVIVAL RATE = $0.56^{1/2}$ = 0.75
   WEEKLY MORTALITY RATE = 1 - 0.75 = 0.25

Method B. Egg counting

1. Calculate EGGSURVIVAL for period
   EGGSURVIVAL = TEST EGGS(CORRECTED) / ORIGINAL EGGS
   TEST EGGS(CORRECTED) = total number eggs retrieved from rings by sieving and bleaching X the efficiency of the sieving and bleaching method (0.90 retrieved).
   TEST EGGS(CORRECTED) = 79 X 0.90 = 87.8
   EGGSURVIVAL = 87.8 / 150 = 0.58

2. Calculate WEEKLY MORTALITY RATE
   WEEKLY MORTALITY RATE = 1 - WEEKLY SURVIVAL RATE
   WEEKLY SURVIVAL RATE = EGGSURVIVAL raised to the 1/T power where T is the exposure period in weeks.
   WEEKLY SURVIVAL RATE = $0.58^{1/2}$ = 0.76
   WEEKLY MORTALITY RATE = 1 - 0.76 = 0.24

APPENDIX D
COMPREHENSIVE LIST OF ANIMALS EXPOSED TO AEDES TAENIORHYNCHUS EGGS IN THE LABORATORY

Test animal

Phylum Arthropoda
Class Crustacea

A. Order Amphipoda
   1. Talitrus specificus (Hurley)
B. Order Decapoda
   1. Sesarma ricordi H. Milne Edwards
   2. Uca rapax (Smith)
B. Order Diplopoda
   1. unidentified
C. Order Isopoda
   1. Porcellio virgatus (Budde-Lund)
   2. Tylos niveus Budde-Lund

Class Insecta

A. Order Collembola
   1. unidentified (20 specimens)
B. Order Orthoptera
   Family Gryllidae
   1. Neonemobius cubensis (Saussure)
   Family Blattidae
   1. unidentified (4 specimens)
C. Order Coleoptera
   Family Carabidae
   1. Loxandrus sp. (larva)
   2. Loxandrus rectangulus LeConte
   3. Oodes amaroides Dejean
   4. Scarites sp. (larva)
   Family Staphylinidae
   1. Achenomorphus corticinus (Gravenhorst)
   2. Acylophorus princeps Smetana
   3. Acylophorus sp.
   4. Astenus sp.
   5. Carpelimus maculicollis (Notman)
   6. Carpelimus sp.
   7. Manda nearctica Moore
   8. Myllaena insipiens Casey
   9. Neobisnius ludicrus (Erichson)
   10. Neohypnus pusillus (Sachse)
   11. Philonthus alumnus Erichson

12. Pinophilus sp. (larva and adult)
13. Scopaeus elaboratus (Casey)
14. Scopaeus sp.
15. Stamnoderus pallidus Casey
16. Subfamily Paederinae (larva)

D. Order Hymenoptera
Family Formicidae
1. Camponotus abdominalis floridanus (Buckley)
2. Hyponera opaciceps (Mayr)
3. Paratrechina bourbonica (Forel)

Phylum Mollusca
1. Ellobium pellucens (Menke)
2. Melampus coffeus (Linne)
3. Polygyra coreolus (Muhlfeldt)

## APPENDIX E
## LINE LISTING OF TAENISIM

Note: Lines 3000 and above are SYSTEM DYNAMICS by Roberts et al. (1983b).

```
1 rem model version based on Dogwood data: ARRAY VERSION
5 rem incorporates 8 topographical strata for egg
   desposition and hatch
10 rem contains 3 strata for simultaneous tracking of
   overlapping
11 rem larval and adult populations
20 GOSUB 3000
30 rem number of table functions and 3rd-order delays
35 DATA 0,0
40 DIM RAN(400),NTBL(400),TDE(400),WEEK(400)
42 rem dimension arrays
43 DIM MATEGG(3,8),IMMVIABLEGG(3,8),IMMEGGMORT(3,8)
44 DIM COVIPOS(3,8),SUBIMMEGG(3,8),POVIPOS(3,8)
50 rem initial values for late spring start
51 rem set proportionate area for each stratum
52 PROPAREA(1)=0.637:PROPAREA(2)=0.924:PROPAREA(3)=1.327:
   PROPAREA(4)=1.243
53 PROPAREA(5)=0.945:PROPAREA(6)=0.684:PROPAREA(7)=0.733:
   PROPAREA(8)=1.507
55 SUMPROPAREA=8:STRATA=1:RAIN=0:A=2:B=.5:C=1.1:D=0.9
57 FOR N= 1 TO 3 : FOR I = 1 TO 8
60      IMMVIABLEGG(N,I)=  0 : NEXT I:NEXT N
69 rem egg values for standard start
70 MATVIABLEGG(1)=05:MATVIABLEGG(2)=20:MATVIABLEGG(3)=50:
   MATVIABLEGG(4)=30
71 MATVIABLEGG(5)=20:MATVIABLEGG(6)=10:MATVIABLEGG(7)=8:
   MATVIABLEGG(8)=5
80 LARVPOP=0:FOR I=1 TO 3:LARVPOP(I)=0:LARVTIME(I)=0:NEXT I
85 rem adult females start at value that produces ca. 20
   eggs/ strata
86 rem 20 females = 20/8 or 2.5/unit strata; unit defined
   as 10 cm diameter
87 rem circle, i.e. the size of 1 sod sample.  These
   mosquitoes laid
88 rem a max. egg population of 177 or 177/8 = 22.125
   eggs/sod
89 rem incorporating a hatch of ca. 90% = 19.9 eggs/sod.
90 rem this correlates well with mean late spring
   populations.
91 ADULTFEM(1) =50:BFED=0.15:AUTOGEN=1-BFED:
100 GRAVTIME(1)=0:GRAVTIME(2)=0:GRAVTIME(3)=0
101 rem eggbatch is # eggs/gonotrophic cycle * fertility
```

```
102 rem egg batches for both bloodfed and autogenous eggs
    included
103 rem eggbatch from field data; autoegg from O'Meara
105 EGGBATCH=82*.914:AUTOEGG=25*.914:MEANEGGPOP=EGGPOP/8
106 rem set initial late spring hydrologic values
110 NEWTABLE=1.6:OLDTABLE=1.6:LARVTIME(1)=0:LARVTIME(2)=0:
    LARVTIME(3)=0
111 rem set elevation values for strata
112 FOR I = 1 TO 7:ELEV(1)=1.6:ELEV(I+1)=ELEV(I)+0.2:NEXT I
113 rem more watertable constants; final model will have
    read statement
114 FLOODTIME = 0:EVAPOTRANS = 0.004
130 rem time parameters
140 T0=.01
150 T1=18
160 T2=53
170 T3=.5:rem a day period
175 GOSUB 2500
180 FOR T=T1 TO T2 STEP T0
182 REM INTRODUCTIONS OF MIGRANTS
185 IF T > 18 AND t < 18.1 THEN ADULTFEM(1)=70
190 rem set variables for input data
191 DATACOUNT = DATACOUNT + T0:REM IF T>=.5 THEN T3=1/7
192 IF DATACOUNT > 1/7 THEN GOTO 193 ELSE GOTO 200
193 DATCNT% = DATCNT% + 1
195 RAIN = RAN(DATCNT%) : NEWTABLE = NTBL(DATCNT%) : TIDE =
    TDE(DATCNT%)
196 DATACOUNT = 0
200 rem set timer if basin flooded (i.e. > 0.2 ft water).
204 rem this value used in larval predation model
205 IF NEWTABLE >1.8 THEN FLOODTIME = FLOODTIME+T0 ELSE
    FLOODTIME=0
206 IF TIDE > 3.2 THEN TIDEFLOOD = 1
207 IF NEWTABLE < 1.8 THEN TIDEFLOOD = 0
208 IF TIDEFLOOD > 0 THEN TFLOODTIME = TFLOODTIME + T0 ELSE
    TFLOODTIME=0
211 rem flood value > 0.2 indicates that flooding has
    occurred
212 rem so goto hatching subroutine (gosub 1400)
213 IF RAIN > 0.5 THEN GOSUB 1400 ELSE IF TIDE > 3.2  THEN
    GOSUB 1400
221 rem if a stratum dries, unhatched eggs are
    reconditioned (gosub 1800)
222 FLOOD=NEWTABLE-OLDTABLE:DRY = DRY + FLOOD
223 IF DRY < -0.2 THEN GOSUB 1800
225 rem next line resets watertable value
226 OLDTABLE=NEWTABLE
227 rem rate equations
230 rem oviposition rate is # gravid mosquitoes X eggbatch
231 rem for each gonotrophic cycle (PREOVIPOSTIME), a
    timer, (GRAVTIME)
232 rem is set for each brood to track oviposition
```

```
233 rem PREOVIPOSTIME is based on a sin function reflecting
    temperature.
234 rem when GRAVTIME > PREOVIPOSTIME, a brood will
    oviposit.
235 OVIPOS=0:FOR I=1 TO 3:OVIP(I)=0:NEXT I
236 FOR I = 1 TO 3
240 IF ADULTFEM(I) > 0 THEN 242 ELSE 283
242 IF FEMEMERGE(I) > 1 THEN 243 ELSE 244
243 MATETIME(I)=2/7 : GRAVTIME(I)=0 :
    FEMEMERGE(I)=0:ADULTEMERGE=0
244 IF GRAVTIME(I) = 0 THEN 249 ELSE 251
249 PREOVIPOSTIME(I)=(1.1+0.9*SIN(6.285*(T+10)/52))
250 IF PREOVIPOSTIME(I) < 0.6 THEN PREOVIPOSTIME(I) = 0.6 +
    MATETIME(I)
251 GRAVTIME(I)=GRAVTIME(I)+T0
255 IF GRAVTIME(I) > PREOVIPOSTIME(I) THEN 262 ELSE GOTO
    283
256 rem note that only 15% of available females
    bloodfeed/week (O'Meara)
257 rem for the 1st gonotrophic cycle; the remainder
    produce
258 rem eggs autogenously  (eggbatch = 25).  For next
    gonotrophic
259 rem cycle, all supposedly bloodfeed
260 rem GONOCYCLE refers to number of gontropohic
    cyles/brood;
261 rem at values above 1, all females bloodfeed
    (O'Meara, PC)
262 GONOCYCLE(I) = GONOCYCLE(I)+1
263 IF GONOCYCLE(I) > 1 THEN GOTO 264 ELSE 265
264 BFED=.80 : AUTOGEN=0 : GOTO 267
265 BFED = 0.15 : AUTOGEN = 0.85
267 OVIP(I)=((ADULTFEM(I)*BFED*EGGBATCH)+(ADULTFEM(I)
    *AUTOGEN*AUTOEGG))/T0
270 OVIPOS=OVIPOS+OVIP(I): GRAVTIME(I)=0:GOTO 1140
283 MATETIME(I)=0:NEXT I
285 FOR N = 1 TO 3:FEMEMERGE(I)=0
286 IF ADULTFEM(N) < .1 THEN GONOCYCLE(N) = 0
300 rem egg maturation timer (EGGMATURETIME) set
301 IF IMMEGGPOP(N) > 0 THEN EGGMATURETIME(N) =
    EGGMATURETIME(N) + T0
304 rem egg maturation rate is a seasonal based sin
    function
305 IF EGGMATURETIME(N) > T0 THEN 308
307 EGGMAT = (1.1 + 0.9*SIN(6.283*(T+10)/52))
308 IF EGGMAT < (3/7) THEN EGGMAT = (3/7)
309 IF EGGMATURETIME(N) >= EGGMAT THEN GOTO 313 ELSE 318
313 FOR E = 1 TO 8: S = 1
314       MATEGG(N,E)=IMMVIABLEGG(N,E)
```

```
316       NEXT E : EGGMATURETIME(N) = 0
318 NEXT N
320 rem immature egg mortality same as mature egg mortality
322 rem based on field studies at Dogwood; sites w/o
    pesticide
323 rem may have higher rates
324 rem value also sin function due to seasonal affects
325 EGGSURVIVE=(.6+0.2*SIN(6.283*(T+10)/52)):EGGMORT=(1-
    (EGGSURVIVE^T0))
326 rem submerged egg mortality is less than exposed eggs?
327 rem guessing (based on limited data) 20%/week
328 SUBEGGMORT = 1-((1-0.20)^T0)
335 FOR E = 1 TO 8
340     FOR N = 1 TO 3
350       IMMEGGMORT(N,E)=IMMVIABLEGG(N,E)*EGGMORT
351     NEXT N
352       SUBEGGMORT(E)=SUBMATEGG(E)*SUBEGGMORT
450       MATUREGGMORT(E)=EGGMORT*MATVIABLEGG(E)
460 NEXT E
500 rem percent hatch varies with season (diapause in
    winter)
510 rem hatch is instantaneous when a strata is flooded.
518 PHATCH = (1.3+SIN(6.283*(T-11)/52))
520 IF PHATCH > 0.95 THEN PHATCH = 0.95
521 rem sumhatch represents cumulative hatch for all strata
526 rem reset hatches to 0
527 FOR L = 1 TO 8 : HATCH(L)=0:NEXT L
531 rem simultaneous larval population array, assings tag
    of 1-3
532 rem to overlapping larval populations.  Three should
    suffice for
533 rem summer since larvae emerge within 1 week
534 rem the following is a bypass if no hatch has occurred
540 IF SUMHATCH = 0 THEN 570
545 rem calculation of time to emergence (EMERGETIME);
    based on
546 rem seasonal mean temperature plus 2 degrees C (water
    temp.)
548 EMERGETIME=(SIN(6.283*(T+10)/52)*0.7+1.35)
549 IF EMERGETIME <= .90 THEN EMERGETIME = .90
550 FOR I=1 TO 3
551 rem larval survival based on flood source and duration
552 rem survival for rain flood
553 IF TIDEFLOOD=0 THEN
    FLARVMORT(I)=((0.4+(0.06*FLOODTIME)))
554 IF FLARVMORT(I) > 0.9 THEN FLARVMORT(I) = 0.90
555 rem survival for tide flood is lower due to fish input
556 IF TIDEFLOOD >=1 THEN
    TLARVMORT(I)=(0.50+(0.15*TFLOODTIME))
557 IF TLARVMORT(I)>0.95 THEN TLARVMORT(I) = 0.95
```

```
558 IF TLARVMORT(I) > FLARVMORT(I) THEN 559 ELSE
    LARVSURVIVAL(I)=1-FLARVMORT(I):GOTO 560
559 LARVSURVIVAL(I) = 1 - TLARVMORT(I)
560 IF LARVPOP(I)<>0 THEN NEXT I :
    LARVTIME(I)=LARVTIME(I)+T0
570 LARVHATCH(I)=SUMHATCH*LARVSURVIVAL(I)
620 IF LARVTIME(1)>=EMERGETIME THEN 630 ELSE 621
621 IF LARVTIME(2)>=EMERGETIME THEN 631 ELSE 622
622 IF LARVTIME(3)>=EMERGETIME THEN 632 ELSE 650
630 LARVTIME(1)=0:GOTO 640
631 LARVTIME(2)=0:GOTO 641
632 LARVTIME(3)=0:GOTO 642
640 ADULTEMERGE(1)=LARVPOP(1)/1:GOTO 650
641 ADULTEMERGE(2)=LARVPOP(2)/1:GOTO 660
642 ADULTEMERGE(3)=LARVPOP(3)/1:GOTO 670
650 LARVPOP(1)=LARVPOP(1)-ADULTEMERGE(1)+T0*(LARVHATCH(1))
660 LARVPOP(2)=LARVPOP(2)-ADULTEMERGE(2)+T0*(LARVHATCH(2))
670 LARVPOP(3)=LARVPOP(3)-ADULTEMERGE(3)+T0*(LARVHATCH(3))
672 FOR I = 1 TO 3:
    LARVTEST(I)=LARVPOP(I)*(0.8*LARVSURVIVAL(I))
673 LARVTEST = LARVTEST + LARVTEST(I):NEXT I
674 XLARVTEST=LARVTEST/STRATA
675 rem reset larvhatch
678 LARVHATCH(I)=0
680 IF LARVPOP(1)>0 THEN LARVTIME(1)=LARVTIME(1)+T0 ELSE
    LARVPOP(1)=0
690 IF LARVPOP(2)>0 THEN LARVTIME(2)=LARVTIME(2)+T0 ELSE
    LARVPOP(2)=0
700 IF LARVPOP(3)>0 THEN LARVTIME(3)=LARVTIME(3)+T0 ELSE
    LARVPOP(3)=0
701 rem larvpop is total for all LARVPOP(I)'S
710 LARVPOP=LARVPOP(1)-LARVPOP(2)+LARVPOP(3)
720 rem adult females mortality is seasonal too
721 rem weekly mortality of 10 - 50% based on summer values
    of
722 rem 15%/day (MARK-RECAPTURE DATA PROVOST (NAYAR, PERS.
    COMM.)
723 REM THIS IS 0.0188/0.01 WEEK
724 ADULTSURVIVAL=(0.4+0.2*SIN(6.283*(T+10)/52))
727 ADULTMORT=1-(ADULTSURVIVAL ^ T0)
730 FOR F=1 TO 3:ADULTFEMORT(F)=ADULTMORT*ADULTFEM(F):
    NEXT F
731 rem no gravid female level since gravids grouped with
    nongravids
770 rem plot and print variables
771 rem fix command rounds to nearest integer, avoiding
    table overrun
800 X(1)=(FIX(100*T))/100
801 X(2)=(FIX(100*NEWTABLE))/100
805 X(3)=(FIX(100*ADULTFEM(1)))/100
```

```
810 X(4)=(FIX(100*ADULTFEM(2)))/100
820 X(5)=(FIX(100*ADULTFEM(3)))/100
825 rem reset variables used in hatching and egg dynamics
subroutines
826 IMMEGGPOP=0:EGGPOP=0:TOTHATCH=0:FLUSHLARVUP=0:
    FLUSHLARVLO=0:SUMHATCH=0
827 SUBMATEGGPOP=0:OVIPOS=0:TIDE = 0:SUBIMMEGGPOP=0:
    LARVTEST=0:RECONDEGGPOP=0
828 FOR N=1 TO 3:IMMEGGPOP(N)=0:NEXT N
830 GOSUB 3100
831 rem
832 rem
833 rem
840 rem level equations
850 FOR EL = 1 TO 8
855   FOR N = 1 TO 3 : S=1
860     rem population of immature viable eggs
870     IMMVIABLEGG(N,EL)=IMMVIABLEGG(N,EL)-MATEGG(N,EL)-
        IMMEGGMORT(N,EL)+T0*(COVIPOS(N,EL)-SUBIMMEGG(N,EL))
872     IF IMMVIABLEGG(N,EL) < 0 THEN IMMVIABLEGG(N,EL) = 0
877     MATEGGS(S,EL)=MATEGGS(S,EL)+MATEGG(N,EL)
878     IMMEGGPOP(N)=IMMEGGPOP(N)+IMMVIABLEGG(N,EL)
879     IMMEGGPOP=IMMEGGPOP+IMMVIABLEGG(N,EL)
880     IF IMMVIABLEGG(N,EL) < 0 THEN IMMVIABLEGG(N,EL)=0
881     SUBIMMEGG(S,EL)=SUBIMMEGG(S,EL)+SUBIMMEGG(N,EL)
890   NEXT N:NEXT EL
910 rem mature viable egg level; note hatche(s) reset to 0
915 FOR EL = 1 TO 8
920 MATVIABLEGG(EL)=MATVIABLEGG(EL)+MATEGGS(S,EL)-
    MATUREGGMORT(EL)+T0*(RECONDEGG(EL)-SUMHATEGG(EL)-
    NOHATCH(EL))
921 SUMHATEGG(EL)=0:FLUSHATCHLO(EL)=0:FLUSHATCHUP(EL)=0
962 rem RECONDEGGS are flooded, unhatched eggs exposed
    after drydown
970 SUBMATEGG(EL)=SUBMATEGG(EL)-SUBEGGMORT(EL)+
    T0*((NOHATCH(EL)+SUBIMMEGG(S,EL))-RECONDEGG(EL))
980 rem set egg levels to 0 if less than 0
982 IF MATVIABLEGG(EL) < 0 THEN MATVIABLEGG(EL) = 0
983 IF SUBMATEGG(EL) < 0 THEN SUBMATEGG(EL) = 0
985 rem calculate cumulative egg population for all strata
990 RECONDEGGPOP= T0*(RECONDEGGPOP + RECONDEGG(EL))
992 EGGPOP=EGGPOP + MATVIABLEGG(EL)
993 SUBMATEGGPOP=SUBMATEGGPOP + SUBMATEGG(EL)
998 REM MEANEGGPOP=EGGPOP/(SUMPROPAREA):SUMPROPAREA=0
1000 rem reset NOHATCH, SUBEGGMORT, MATEGG, and RECONDEGG
    to 0
```

```
1001 rem until values assigned by HATCH SUBROUTINE
1002 NOHATCH(EL)=0:SUBEGGMORT(EL)=0:RECONDEGG(EL)=0:
     SUBIMMEGG(S,EL)=0
1003 MATEGGS(S,EL)=0
1004   FOR N = 1 TO 3 : MATEGG(N,EL)=0 :COVIPOS(N,EL)=0:
       POVIPOS(N,EL)=0
1005   NEXT N
1006 NEXT EL
1010 rem adult female population level; broods 1, 2 and 3
1031 rem time to mate before bloodfeeding and egg
     development = 2 days
1032 rem tag a value of 2 days to PREOVIPOSTIME when
     females emerge
1033 rem reset adultfem (cumulative population) to 0 then
     recalculate
1034 ADULTFEM = 0
1035 FOR F = 1 TO 3 :
1036 ADULTEMERGE=ADULTEMERGE+ADULTEMERGE(F):FEMEMERGE(I)=0
1037 NEXT F
1038 rem adjust emerging females for larval survival and
     sex ratio (-50%)
1039 IF ADULTEMERGE > 0 THEN GOTO 1040 ELSE GOTO 1046
1040 FOR E = 1 TO 4
1041    IF ADULTFEM(E) < 1 THEN 1042 ELSE NEXT E
1042    IF E=4 THEN FEMEMERGE(1) = ADULTEMERGE*.5:GOTO 1046
1045    FEMEMERGE(E)=ADULTEMERGE*.5:GOTO 1046
1046 FOR F = 1 TO 3
1048    ADULTFEM(F)=ADULTFEM(F)+FEMEMERGE(F)-ADULTFEMORT(F)
1050    LARVHATCH(F)=0:LARVTEST(F)=0
1051    ADULTFEM = ADULTFEM +ADULTFEM(F)
1058    ADULTEMERGE(F)=0
1060 NEXT F
1061 ADULTEMERGE = 0
1080 NEXT T
1090 rem data for graphs; levels for mature viable eggs,
     larvae and adult
1100 rem female populations
1110 DATA 5, T, W, E, L, A
1120 DATA  0, 60, 0, 4, 0, 2000, 0, 1600, 0, 500
1130 STOP
1140 rem oviposition subroutine; based on elevational
     strata
1150 rem oviposition strata and egg elevation settings
1155 C=0:PROPAREA=0:SUMOVIPOS=0:SUMCOVIPOS=0
1158 FOR N = 1 TO 3
1159 IF OVIP(N) = 0 THEN NEXT N
1160 FOR I = 1 TO 8
1165    IF ELEV(I) > NEWTABLE THEN GOTO 1170 ELSE 1195
```

```
1170 POVIPOS(N,I)=100/(1+(0.02/(NEWTABLE/ELEV(1))*(ELEV(I)-
     (NEWTABLE+.1))^-3))
1175    IF POVIPOS(N,I) < 0 THEN POVIPOS(N,I) = 0
1178    POVIPOS(N,8)=100
1180    OVIPOS(N,I)=((POVIPOS(N,I)-POVIPOS(N,I-1))/100)
        *OVIPOS*PROPAREA(I)
1185    rem counter for correction figure for relative
        strata area
1192    SUMOVIPOS =SUMOVIPOS + OVIPOS(N,I)
1195 NEXT I
1200 rem loop for calculation of oviposition corrected for
     relative area
1202 FOR I = 1 TO 8
1204    IF ELEV(I) > NEWTABLE THEN GOTO 1206 ELSE 1209
1206    COVIPOS(N,I)=OVIPOS(N,I)*(OVIPOS/SUMOVIPOS)
1207    SUMCOVIPOS=SUMCOVIPOS + COVIPOS(N,I)
1209 NEXT I
1210 GOTO 285
1400 rem hatch subroutine; based on elevational strata
1402 IF NEWTABLE <= 1.9 THEN STRATA = 1:GOTO 1630:
     REM 1.7 - 1.9 STAGE
1403 IF NEWTABLE <= 2.1 THEN STRATA = 2:GOTO 1630:
     REM 1.9 - 2.1 STAGE
1405 IF NEWTABLE <= 2.3 THEN STRATA = 3 :GOTO 1630:
     REM 2.1 - 2.3 STAGE
1406 IF NEWTABLE <= 2.5 THEN STRATA = 4 :GOTO 1630:
     REM 2.3 - 2.5 STAGE
1407 IF NEWTABLE <= 2.7 THEN STRATA = 5 :GOTO 1630:
     REM 2.5 - 2.7 STAGE
1408 IF NEWTABLE <= 2.9 THEN STRATA = 6 :GOTO 1630:
     REM 2.7 - 2.9 STAGE
1409 IF NEWTABLE  > 2.9 THEN STRATA = 7 :GOTO 1630:
     REM 2.9+ STAGE
1620 rem three hatching processes are outlined below;
1621 rem 1. flood hatching (eggs inundated by standing
     water)
1622 rem 2. lowland flush hatching (lowland Dogwood eggs
     exposed to
1623 rem    runoff
1624 rem 3. upland flush hatching (upland Dogwood eggs
     exposed to runoff
1629 rem flood hatch subroutine
1630    FOR H = 1 TO STRATA
1631    HATCH(H) = (MATVIABLEGG(H) * PHATCH)/T0
1632    NOHATCH(H) = (1 - PHATCH)*MATVIABLEGG(H)/T0
1633    TOTHATCH = TOTHATCH + HATCH(H)
1634    SUBEGGMORT(H) = (SUBMATEGG(H) * 0.2)/T0
1635      FOR N = 1 TO 3:SUBIMMEGG(N,H) =
          IMMVIABLEGG(N,H)/T0:NEXT N
```

```
1638     NEXT H
1640 rem lowland flush subroutine
1641     FOR FHLO = (STRATA + 1) TO 6
1642     rem calculate proportion eggs flush hatched
1643     PFLHATLO=(RAIN-0.25)*0.27:IF PFLHATLO >.20 THEN
         PFLHATLO= 0.20
1644     FLUSHATCHLO(FHLO)=(MATVIABLEGG(FHLO) * PFLHATLO)/T0
1645     rem flush hatch low incorporates % larave reaching
         water
1646     rem assume same relationship as upland
1647     FLARVMORT(FHLO)=(ELEV(FHLO)-NEWTABLE)*0.9
1648     IF NEWTABLE <1.3 THEN LARVMORT(FHLO)=1
1649     FLUSHLARVLO(FHLO)=(1-
         FLARVMORT(FHLO))*FLUSHATCHLO(FHLO)
1650     rem total larvae flushed from low strata
1651     FLUSHLARVLO = (FLUSHLARVLO + FLUSHLARVLO(FHLO))
1652     NEXT FHLO
1660 rem upland flush subroutine
1661     FOR FHUP = 7 TO 8:IF STRATA > 6 THEN FHUP = 8
1662     PROPFLUSHATCHUP=(RAIN-0.25)*1.20:IF RAIN > 1 THEN
         PROPFLUSHATCHUP=0.90
1663     FLUSHATCHUP(FHUP)=(MATVIABLEGG(FHUP) *
         PROPFLUSHATCHUP)/T0
1664     rem survival of larvae flushed is guessed at 70% *
         inverse elevation
1665     FLARVMORT(FHUP)=(ELEV(FHUP)-NEWTABLE)*0.7
1666     IF  NEWTABLE < 1.8 THEN FLARVMORT(FHUP) = 1
1667     FLUSHLARVUP(FHUP) =(1-
         FLARVMORT(FHUP))*FLUSHATCHUP(FHUP)
1668     rem total larvae flushed from upland strata
1669     FLUSHLARVUP=FLUSHLARVUP + FLUSHLARVUP(FHUP)
1670     NEXT FHUP
1671 rem total newly hatched larvae used in LARVPOP
1672 SUMHATCH=TOTHATCH + FLUSHLARVLO + FLUSHLARVUP
1673 rem SUMHATCHEGGS is all eggs hatched and flush-hatched
     from a stratum
1676     FOR HE = 1 TO 8
1677     SUMHATEGG(HE)=(HATCH(HE)+FLUSHATCHLO(HE)+
         FLUSHATCHUP(HE))
1678     NEXT HE
1680 rem reset FLOOD, RAIN and DRY to 0
1681 FLOOD = 0 : RAIN = 0 : DRY = 0 : RETURN
1800 rem reconditioned egg subroutine
1801 IF NEWTABLE <=1.7 THEN RECONDEGG(1)=SUBMATEGG(1)/T0
1802 IF NEWTABLE <=1.9 THEN RECONDEGG(2)=SUBMATEGG(2)/T0
1803 IF NEWTABLE <=2.1 THEN RECONDEGG(3)=SUBMATEGG(3)/T0
1804 IF NEWTABLE <=2.3 THEN RECONDEGG(4)=SUBMATEGG(4)/T0
1805 IF NEWTABLE <=2.5 THEN RECONDEGG(5)=SUBMATEGG(5)/T0
1806 IF NEWTABLE <=2.7 THEN RECONDEGG(6)=SUBMATEGG(6)/T0
```

```
1807 IF NEWTABLE <=2.9 THEN RECONDEGG(7)=SUBMATEGG(7)/TO
1810 DRY = 0 : RETURN
1900 rem good luck; You'll need it!

2500 ' SUBR TO READ DATA INTO PGM BY ED PROFFITT
2501 OPEN"I",#1,"A:TESTDATA.prn"
2504 INPUT "DO YOU WANT TO START AT JANUARY 1?";YN$
2505 IF YN$="Y" THEN CNT=1
2506 IF YN$="y" THEN CNT=1
2507 IF CNT<>1 THEN INPUT "INPUT NUMBER OF WEEK TO
     START>";CNT
2508 PRINT "IF YOU HAVE ANY WEEKS WITHOUT 7 DAYS OF DATA,
     MODEL WILL SCREW UP"
2510 LINE INPUT #1,I$
2520 DATCNT=DATCNT+1
2521 DUM=DUM+1:IF DUM=8 THEN GOTO 2529
2528 IF DUM=7 THEN CNT=CNT+1 ELSE GOTO 2530
2529 DUM=0
2530 RAN(DATCNT)=VAL(LEFT$(I$,9))
2540 NTBL(DATCNT)=VAL(MID$(I$,10,10))
2550 TDE(DATCNT)=VAL(RIGHT$(I$,4))
2560 IF EOF(1) THEN GOTO 2569
2565 GOTO 2510
2569 CNT=1:DUM=1
2570 FOR K=1 TO DATCNT
2571  DUM=DUM+1:WEEK(K)=CNT
2572  IF DUM=8 THEN CNT=CNT+1
2573  IF DUM=8 THEN DUM=1
2575 NEXT K
2580 FOR I=1 TO DATCNT
2585   PRINT WEEK(I),RAN(I),NTBL(I),TDE(I)
2590   NEXT I
2599 RETURN

3000 REM ....SYSTEM DYNAMICS SUBROUTINE PACKAGE, GPR,
     2/4/78
3001 REM SUBROUTINE FOR OUTPUT TO THE SCREEN
3005 DIM T(22,16), X$(9), Z(6,3)
3010 READ Z4 ,Y8: FOR I=1 TO Z4: FOR J=1 TO 3: READ T(I,J):
     NEXT J
3012 IF T(I,3) = 0 THEN PRINT "ZERO INCREMENT IN TABLE"; I:
     STOP
3015 Z = (T(I,2) - T(I,1))/T(I,3)
3017 IF Z -INT(Z)>.000001 THEN PRINT "INCREMENT ERROR IN
     TABLE";I:STOP
3020 FOR J= 4 TO Z+4: READ T(I,J): NEXT J: NEXT I
3025 READ Z1: FOR I=1 TO Z1: READ X$(I): NEXT I:
     Y6=T1:Z5=1: Y9=1
```

```
3030 PRINT "PRINT OR PLOT";: INPUT Z$: Y$=MID$(Z$,1,2):IF
     Y$="PR" THEN 3075
3035 REM ..... SCALES FOR PLOT
3040 PRINT: FOR I=1 TO Z1: READ Z2(I),Z3(I): NEXT I:
     Z7=7:Z8=2:Z9=56
3045 READ W$: IF W$ <> "OK" THEN PRINT "DATA ERROR": STOP
3047 DATA "OK"
3050 IF Y$ = "NS" THEN 3070
3055 FOR I=1 TO Z1: Z2=Z3(I)-Z2(I): PRINT X$(I);"=";
3060 FOR J=0 TO 4: PRINT TAB(Z7-
     Z8+J*Z9/4);Z2(I)+J*Z2/4;:NEXT J
3065 PRINT : NEXT I
3070 PRINT : Z3=10 : RETURN
3075 REM .... HEADINGS FOR PRINT
3080 IF Z1 > 6 THEN PRINT "MORE THAN 6 VARIABLES": STOP
3085 PRINT : FOR Z6= 1 TO Z1: PRINT X$(Z6),:NEXT Z6 : IF Z1
     < 6 THEN PRINT
3090 PRINT : RETURN
3100 IF T < Y6 THEN RETURN
3102 Y6 = T + T3 : IF Y$ = "PR" THEN 3200
3105 REM .... ORDINATES
3115 FOR Z6= 1 TO Z1: Y(Z6)=INT((X(Z6)-Z2(Z6))/(Z3(Z6)-
     Z2(Z6))*Z9+.5): NEXT Z6
3130 REM ....PLOT
3135 IF Z3 = 10 THEN PRINT T; : Z3=0
3140 PRINT TAB(Z7); : FOR Z6 = 0 TO Z9 : FOR Z0= 1 TO Z1
3145 IF Y(Z0)= Z6 THEN PRINT X$(Z0);: GOTO 3170
3150 NEXT Z0: IF 4*Z6/Z9 = INT(4*Z6/Z9) THEN PRINT ".";:
     GOTO 3170
3155 IF Z3 > 0 THEN 3165
3160 IF Z6/2 = INT(Z6/2) THEN PRINT "-";: GOTO 3170
3165 PRINT" ";
3170 NEXT Z6: PRINT: Z3=Z3+1 : RETURN
3200 REM ......PRINT
3205 FOR Z6 = 1 TO Z1 : PRINT X(Z6), : NEXT Z6 : IF Z1 < 6
     THEN PRINT
3215 RETURN
3300 REM .....TABLE SUBROUTINE
3305 IF X<= T(Z5,1) THEN Y = T(Z5,4) :GOTO 3325
3310 IF X>=T(Z5,2) THEN Z=(T(Z5,2)-T(Z5,1))/T(Z5,3) :Y =
     T(Z5,Z+4) : GOTO 3325
3315 Z = INT((X-T(Z5,1))/T(Z5,3))
3320 Y = (T(Z5,Z+5) - T(Z5,Z+4))/ T(Z5,3) * (X-T(Z5,1)-
     Z*T(Z5,3)) + T(Z5,Z+4)
3325 Z5 = Z5 + 1 : IF Z5 > Z4 THEN Z5 =1
3330 RETURN
```

```
3400 REM .... TABLE SUBROUTINE WITHOUT HIGH/LOW CAPABILITY
3405 IF X < T(Z5,1) THEN PRINT "BELOW TABLE"; Z5; "AT T =
     "; : STOP
3410 IF X > T(Z5,2) THEN PRINT "ABOVE TABLE"; Z5; "AT T =
     "; : STOP
3415 GOTO 3315
3500 REM ......THIRD ORDER MATERIAL DELAY SUBROUTINE
3505 Y7 = Y/3  : Z(Y9,0) = X * Y7 : IF T <> T1 THEN 3515
3510 FOR Z0 = 1 TO 3 : Z(Y9,Z0) = Z(Y9,0) : NEXT Z0 : GOTO
     3525
3515 FOR Z0 = 3 TO 1 STEP -1
3520 Z(Y9,Z0) = Z(Y9,Z0) + (T0/Y7) * (Z(Y9,Z0-1)-Z(Y9,Z0))
     : NEXT Z0
3525 Z = Z(Y9,3)/Y7  :  Y9 = Y9+1 : IF Y9 > Y8 THEN Y9 = 1
3530 RETURN
3540 END
```

APPENDIX F
MEASURED ELEVATIONS FROM THE DOGWOOD GRID

X and Y are the grid coordinates in feet and elevation is the elevation in feet above mean sea level.

| X | Y | ELEVATION |
|---|---|---|
| 0 | 0 | 3.4 |
| 0 | 20 | 3.2 |
| 0 | 30 | 3.5 |
| 0 | 35 | 3.3 |
| 0 | 40 | 2.9 |
| 0 | 60 | 2.8 |
| 0 | 80 | 2.5 |
| 0 | 100 | 2.5 |
| 0 | 105 | 2.5 |
| 0 | 120 | 1.9 |
| 15 | 100 | 2.7 |
| 20 | 90 | 2.3 |
| 25 | 0 | 2.8 |
| 25 | 20 | 2.6 |
| 25 | 40 | 2.5 |
| 25 | 60 | 2.5 |
| 25 | 80 | 2.4 |
| 25 | 100 | 2.2 |
| 25 | 120 | 2.5 |
| 30 | 35 | 3.1 |
| 30 | 45 | 2.7 |
| 30 | 85 | 2.7 |
| 30 | 90 | 1.9 |
| 30 | 120 | 2.5 |
| 40 | 105 | 2 |
| 45 | 0 | 2.1 |
| 45 | 20 | 2.2 |
| 45 | 40 | 2.3 |
| 45 | 60 | 2.5 |
| 45 | 80 | 1.9 |
| 45 | 100 | 2.5 |
| 45 | 115 | 2 |
| 45 | 120 | 1.9 |
| 50 | 30 | 1.9 |
| 50 | 55 | 1.8 |
| 50 | 105 | 2.8 |
| 55 | 55 | 1.8 |
| 55 | 70 | 2.8 |
| 55 | 100 | 2.5 |
| 55 | 115 | 2.4 |
| 65 | 0 | 5.2 |

| X | Y | ELEVATION |
|---|---|---|
| 65 | 20 | 1.8 |
| 65 | 25 | 2.7 |
| 65 | 40 | 1.9 |
| 65 | 45 | 1.7 |
| 65 | 60 | 2.1 |
| 65 | 80 | 2.2 |
| 65 | 100 | 1.9 |
| 65 | 105 | 2.5 |
| 65 | 120 | 2 |
| 70 | 70 | 1.4 |
| 75 | 25 | 1.6 |
| 75 | 45 | 2.6 |
| 75 | 50 | 1.5 |
| 80 | 85 | 1.8 |
| 80 | 90 | 2.3 |
| 85 | 0 | 5.9 |
| 85 | 20 | 3 |
| 85 | 40 | 1.6 |
| 85 | 60 | 1.6 |
| 85 | 80 | 2.1 |
| 85 | 100 | 1.8 |
| 85 | 105 | 1.8 |
| 85 | 120 | 2.4 |
| 100 | 95 | 1.5 |
| 105 | 0 | 6.3 |
| 105 | 20 | 2 |
| 105 | 40 | 1.9 |
| 105 | 60 | 1.7 |
| 105 | 80 | 1.6 |
| 105 | 90 | 2 |
| 105 | 100 | 1.3 |
| 105 | 120 | 1.7 |
| 115 | 30 | 2.7 |
| 115 | 90 | 2.6 |
| 125 | 0 | 5.7 |
| 125 | 20 | 4.9 |
| 125 | 40 | 1.6 |
| 125 | 60 | 1.4 |
| 125 | 80 | 1.3 |
| 125 | 100 | 1.6 |
| 125 | 120 | 2.1 |
| 130 | 110 | 1.7 |
| 130 | 120 | 2.6 |
| 135 | 50 | 2 |
| 145 | 0 | 4.8 |
| 145 | 20 | 3.1 |
| 145 | 40 | 2.3 |
| 145 | 60 | 1.4 |
| 145 | 80 | 1.4 |
| 145 | 95 | 1.7 |

| X | Y | ELEVATION |
|---|---|---|
| 145 | 100 | 1.8 |
| 145 | 120 | 2.1 |
| 150 | 70 | 2.7 |
| 155 | 80 | 2.7 |
| 155 | 105 | 2.8 |
| 160 | 0 | 5 |
| 160 | 20 | 3.6 |
| 160 | 40 | 2.3 |
| 160 | 60 | 1.7 |
| 160 | 80 | 2 |
| 160 | 100 | 1.7 |
| 160 | 120 | 1.8 |
| 87 | 47 | 2.4 |
| 100 | 56 | 2 |
| 108 | 60 | 2.3 |
| 95 | 65 | 2.3 |
| 113 | 88 | 2.7 |
| 120 | 92 | 2.5 |
| 124 | 95 | 2.5 |
| 155 | 115 | 2.5 |
| 128 | 75 | 1.1 |
| 132 | 65 | 1.2 |
| 82 | 82 | 2.8 |
| 90 | 80 | 2.2 |
| 80 | 90 | 2.3 |
| 150 | 65 | 1.65 |
| 10 | 115 | 2 |
| 20 | 115 | 2.1 |
| 84 | 80 | 2.6 |
| 86 | 80 | 2.4 |
| 83 | 73 | 1.7 |
| 98 | 65 | 2.2 |
| 92 | 54 | 2.5 |
| 105 | 88 | 1.8 |
| 110 | 90 | 2 |
| 125 | 100 | 2 |

APPENDIX G
LINE LISTING OF HYDROMOD

The following is a listing of the cell formulas used in HYDROMOD in a LOTUS 123® spreadsheet. Letters refer to the column address where specific formulas reside. Numbers refer to the row address where calculations for a specific day reside.

I. Column Headings

```
A3: ^WEEK
B3: "DATE
C3: "MONTH
D3: ^PPT
E3: "TIDE
F3: 'ET
G3: 'CORR ET
H3: 'CHANGE
I3: 'CORR CHG
J3: ^WET MOD
K3: ^DRY MOD
L3: ^FIN MOD
```

II. Column Formulas

```
A5: 13
B5: (D4) @DATE(85,4,1)
C5: @MONTH(B5)
D5: 0
E5: 1.6
F5: (0.04+0.02*@SIN(6.285*((A5-15)/52)-(D5*0.01)))
G5: @IF(F5>0.035,0.035,F5)
H5: @IF(D5>0,(((D5-0.2)*0.3)+(((D5-0.2)^2*0.08)-
    G5)),@IF($L4>0,-G5,(-G5*((1+$L4)^4))))
I5: @IF(H5<=0,H5,@IF(L4<1.6,@IF(H5+$L4<1.6,H5,(1.6-
    $L4)+((H5-(1.6-$L4))*0.1)),H5*0.1))
J5: @IF(E5<3.2,@IF($L4+I5<1.55,$L4+I5,$L4+(I5÷(-
    (0.025*$L4^2)))),@IF(E5-1.5>L4+I5,E5-1.5,L4+I5))
K5: @IF($L4<0,-0.66÷0.2*E5,-5)
```

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## BIOGRAPHICAL SKETCH

Scott A. Ritchie was born one hour late of April Fool's Day in 1956 in Council Bluffs, Iowa. His entomological education was initiated in an abandoned pasture and woodlot adjacent to his house. His formal education includes six years (1974 to 1980) at Iowa State University where he obtained a B.S. (1978) and M.S. (1980) in entomology. He then traveled to Corvallis, Oregon, where he attended Oregon State University for two semesters (1980 to 1981). In 1981, he moved to Naples, Florida, where he was employed as an entomologist by the Collier Mosquito Control District. It was during this employment that Mr. Ritchie was funded by the district for a three-year doctoral program (1985 to 1988) in entomology at the University of Florida. After obtaining his doctoral degree, he plans to continue researching the population dynamics of mosquitoes for the district.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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